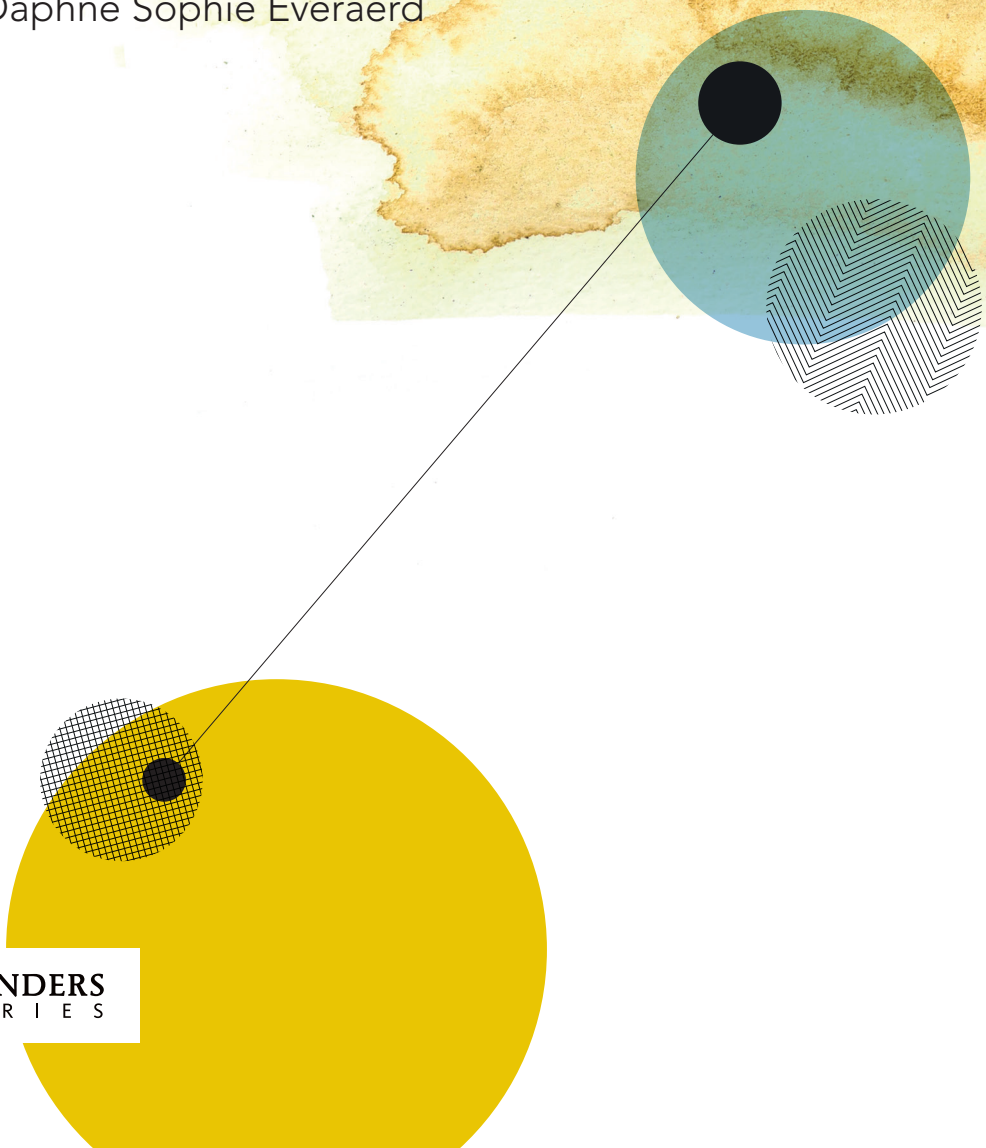
A LOAD ON THE MIND
NEURAL MECHANISMS OF STRESS SENSITIVITY

Daphne Sophie Everaerd



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Neural mechanisms of stress sensitivity

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Neural mechanisms of stress sensitivity

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à chacun son problème

à chacun son affaire

à chacun sa façon

à chacun sa manière

à chacun sa vie

à chacun son affaire

Amadou & Mariam
Sou Ni Tilé, 1999

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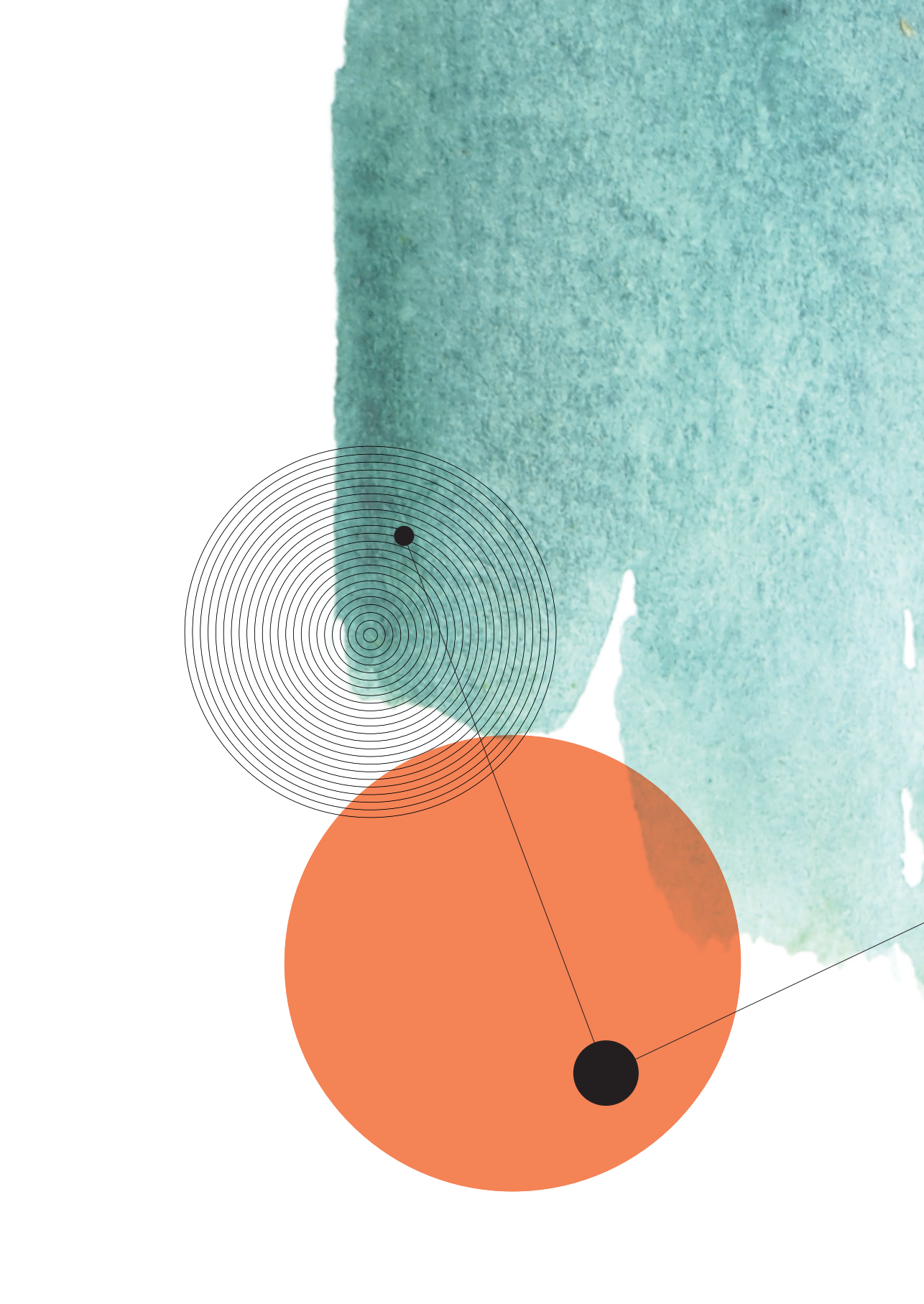
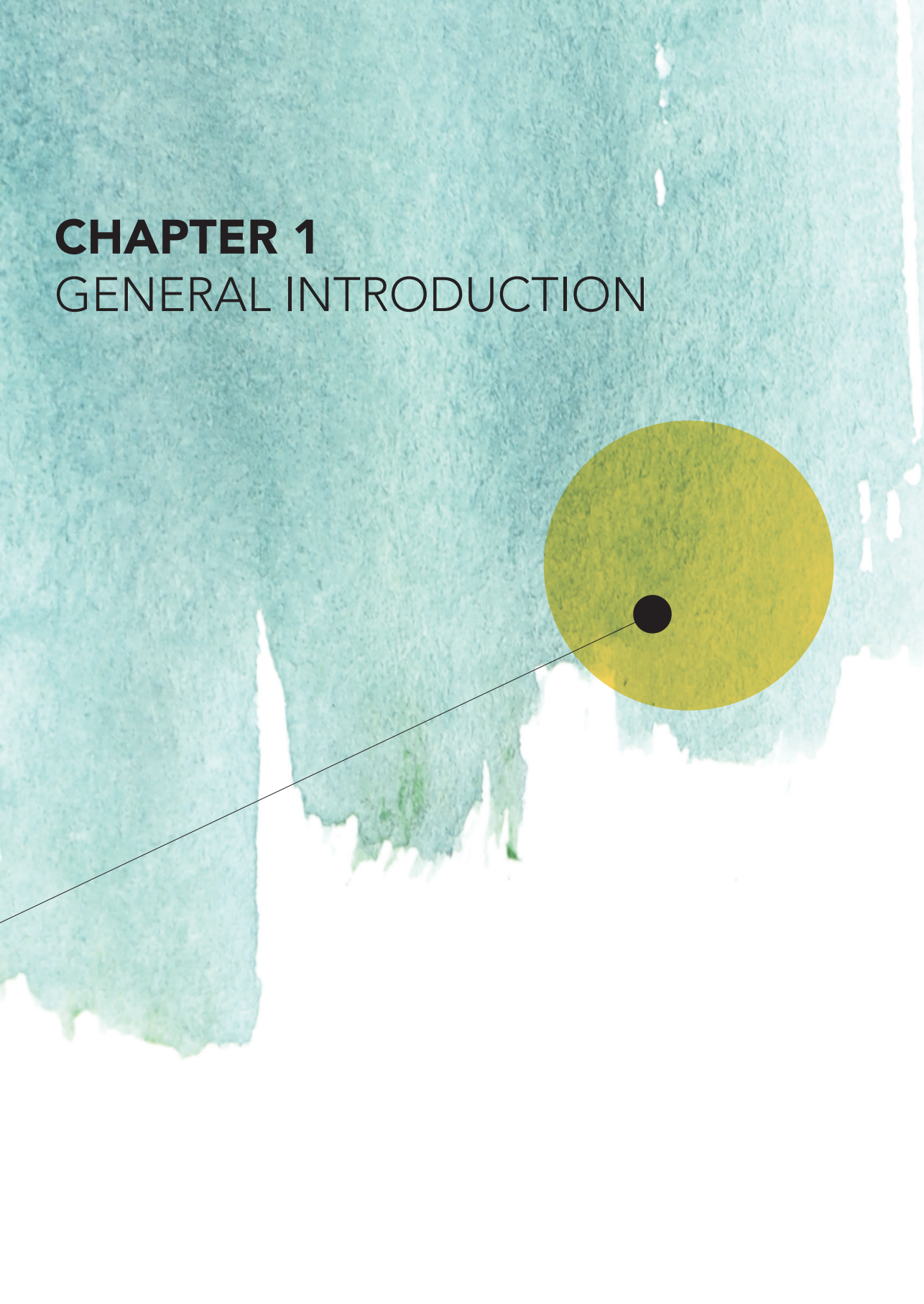


CHAPTER 1
GENERAL INTRODUCTION



1 General introduction

Everyone knows what stress feels like. Think of the despair caused by your little brother running away with your favorite toy. You probably also experienced it in adolescence when you needed to pass an important exam. And throughout adulthood we all still encounter numerous stressors: from getting stuck in a traffic jam to more serious stressors, such as losing work, health problems or the death of a beloved one.

Even though stress has a very general definition and could also be associated with positive effects, it most often has a negative effect on our mood and thoughts. In some cases, stress can even be a cause of mental illness (box 1.1). Therefore, it is important to understand the mechanisms by which stress influences the functioning of our body and brain.

Box 1.1

Stress-related psychiatric disorders

The DSM 5, the diagnostic criteria officially used by psychologists and psychiatrists in the Netherlands, contains a special chapter titled Trauma and Stressor-Related Disorders. In this chapter, the diagnostic criteria of psychiatric disorders that are directly related to a severe traumatic event are described. One example of the disorders included in this chapter is Post Traumatic Stress Disorder (PTSD). In order to fulfill the criteria for PTSD, the patient must have been exposed to death, threatened death, actual or threatened serious injury, or actual or threatened sexual violence (DSM 5, APA).

In contrast to stressor-related disorders, when speaking of stress-related psychiatric disorders, a much larger group of psychiatric disorders is referred to, including mood and anxiety disorders, psychotic disorders and addiction. In fact, most researchers and clinicians will agree that stress is involved in the pathophysiology of nearly all psychiatric disorders. In this sense, stress is not required to take the form of an extremely traumatic experience as in PTSD. It can also be the accumulation of multiple mild stressors leading to, for example, triggering depression onset in a patient who is vulnerable to depression.

From a scientific perspective, the recently developed Research Domain Criteria (RDoC) by the National Institute of Mental Health (NIMH) represent a more complete and constructive classification system (https://www.nimh.nih.gov/research-priorities/rdoc/). The RDoC domains consist of five major systems of emotion, cognition and social behavior: negative valence systems, positive valence systems, cognitive systems, systems for social processes and arousal/ regulatory systems. Each domain reflects a functional construct, consisting of current knowledge on the underlying genes, molecules, cells, circuits, physiology, behavior, self-report and paradigms. The aim of the development of these criteria is to provide more effective diagnosis for patients in the future (Insel, 2010). Stress is most directly represented in the arousal/ regulatory system, but also interacts closely with other systems, for example in fear and anxiety (negative valence systems) or when stress influences decision making (positive valence systems), memory (cognitive systems) and social communication (systems for social processes). Therefore, the RDoC domains and constructs provide a useful framework for future stress research.

While stress is a daily phenomenon for most of us and most likely also for our ancestors, the use of the word 'stress' as to refer to biological or psychological strain is quite recent. One of the first people to describe this type of stress was the endocrinologist Hans Selye, who described that when administering unspecific harmful agents to an organism: "a typical syndrome appears, the symptoms of which are independent of the nature of the damaging agent or the pharmacological type of the drug employed, and represent rather a response to damage as such" (Selye, 1936). Years later John Mason described a more psychological type of stress. He found that during stress reactions in experiments the factors of novelty, uncertainty, or unpredictability can have particularly potent influences on the stress response, therefore constituting an essential component of a stressor (Mason, 1968). More recent seminal work by Bruce McEwen and colleagues has introduced stress as anything disrupting an organism's homeostasis (equilibrium), creating a process of allostasis: the active process during which an organism tries to adapt to these stressors to maintain homeostasis (McEwen, 1998; McEwen, Bowles, Gray, & Hill, 2015). Although responses to stress are most often adaptive, excess stress can lead to allostatic overload and result in maladaptation and psychopathology (McEwen, 1998; McEwen et al., 2015).

1.1 Variation in stress vulnerability

Events that cause stress differ from person to person. One reason for this variation is that the same type of stressor can have a different impact on different people, depending on factors such as sex, age and personality traits. For example, while some drivers may get very agitated when stuck in a traffic jam, others will just turn on the radio and stay calm. More importantly, while some people will experience no long-lasting effects on their mood after a more serious stressor such as the death of a beloved one, others will develop a mood disorder such as major depressive disorder. Understanding these individual differences in stress vulnerability and resilience is crucial for gaining better insight into the mechanisms leading to adaptive responses to stress versus stress-related psychopathology. Below some well-known factors that can influence the impact of a stressor on our brain and behavior will be discussed.

Sex

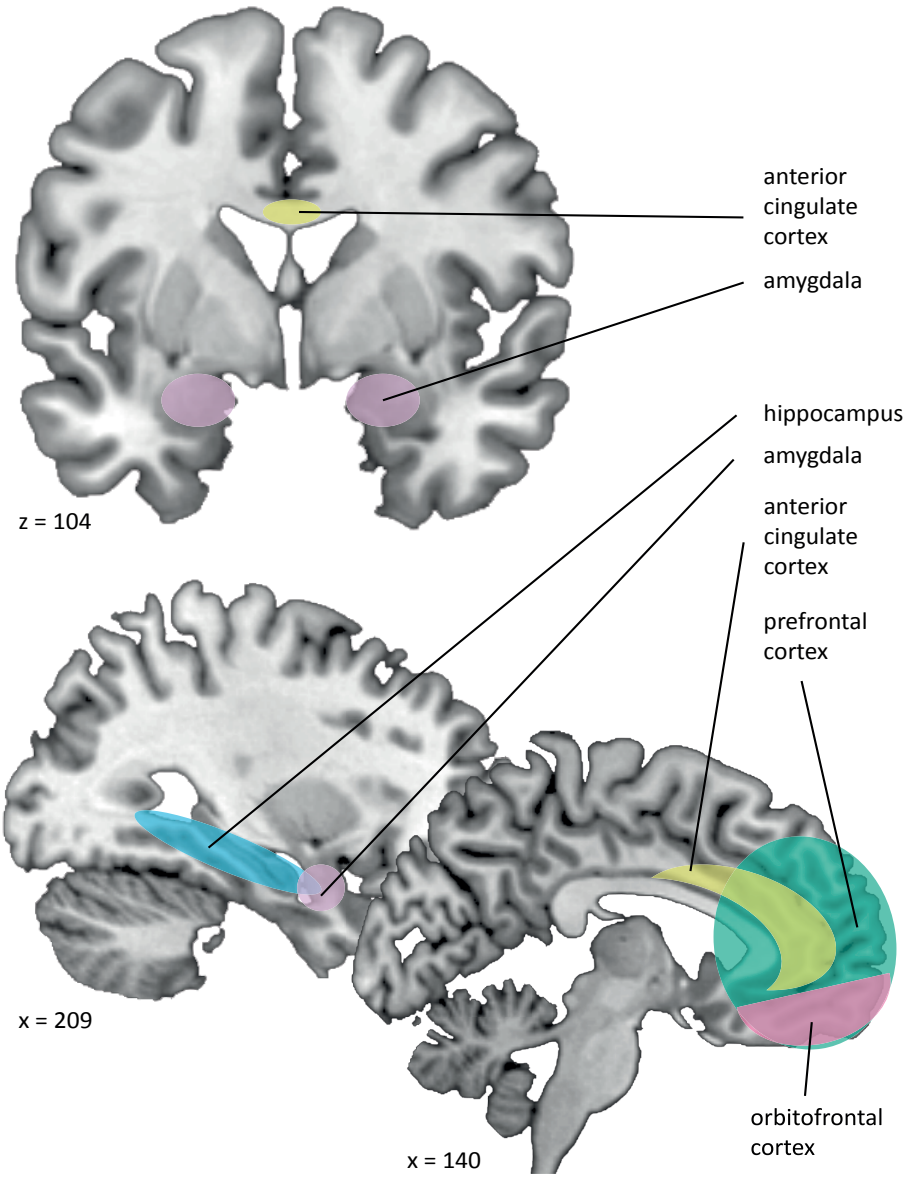
Since depressive disorders and anxiety disorders are more common in women than men (Kessler et al., 2005), sex differences in stress responses have been an important topic of investigation (Cahill, 2006; Kessler et al., 2005). Differences between men and women exist at different levels of the stress response. Firstly, stress hormone responses to a laboratory stressor are different between men and women, and even differ between women in different phases of their menstrual cycle (Kudielka & Kirschbaum, 2005; Young & Korszun, 2010). Interestingly, cortisol responses to stress in

adult men are higher than in adult women, although this may depend on the specific paradigm used (Kudielka & Kirschbaum, 2005; Merz & Wolf, 2017). Sexual dimorphism is also present at the brain level, with significant sex differences in the anatomy of brain regions involved in stress, as well as neurotransmitter receptor distributions (Cahill, 2006). While some differences are the result of gonadal hormone effects during development, other sex differences are thought to arise from more fundamental genetic and epigenetic mechanisms (Bale & Epperson, 2015; Jazin & Cahill, 2010). Finally, research in opposite sex twin pairs has demonstrated that even with a similar genetic profile, different types of stressors can have a dissimilar impact on the etiological pathways to depression in men and women (Kendler & Gardner, 2014).

Age

Childhood and old age are periods in life during which the brain is particularly sensitive to influences from the environment, providing windows of vulnerability for the damaging effects of stress. These windows probably exist because the brain undergoes many changes during these periods (Lupien, McEwen, Gunnar, & Heim, 2009). In addition, the aging brain has had much time to accumulate chronic stressors which may add up to even more vulnerability in combination with less ability for recovery (McEwen & Morrison, 2013; McEwen, Nasca, & Gray, 2016; Prenderville, Kennedy, Dinan, & Cryan, 2015). Correspondingly, mood and anxiety disorders are very common in late life. A recent study amongst community dwelling adults of 55 years and older confirmed that stress-related psychiatric disorders remained very frequent, with 12-month prevalence rates of 4.9% for any mood disorder and 11.6% for any anxiety disorder (Byers, Yaffe, Covinsky, Friedman, & Bruce, 2010). Bereavement, medical illness and disability are prevalent stressors at older age and have been identified to be important psychosocial risk factors for depression in the elderly (Bruce, 2002; M. G. Cole & Dendukuri, 2003).

Contrary, there is also evidence that the healthy aging brain may mediate resilient behavioral traits: healthy older adults, at least in Western countries, are more effective in regulating negative emotions than young adults, contributing to better emotional well-being in daily life (Brassen, Gamer, Peters, Gluth, & Büchel, 2012; Mather, 2012; 2016; Steptoe, Deaton, & Stone, 2015). This phenomenon has received much attention and is called the “positivity effect” (Carstensen, 2006; Carstensen & Mikels, 2005; Mather & Carstensen, 2005; Scheibe & Carstensen, 2010). With respect to the underlying neurobiology, there are two opposing theoretical accounts of this positivity effect. The aging-brain model (Cacioppo, Berntson, Bechara, Tranel, & Hawkley, 2011) suggests that older adults` positivity effect is a consequence of age-related functional decline in the amygdala. The amygdala is important for the identification of the emotional significance of fearful environmental stimuli, the production of affective states, as well as


anterior
cingulate
cortex
amygdala
z = 104
hippocampus
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prefrontal
cortex
x = 209
x = 140
orbitofrontal
cortex

Figure 1.1 Key brain regions involved in emotion processing.
The amygdala plays a central role for emotional influences on attention and perception, emotional learning and memory, emotional behavior and emotion regulation (Phelps & LeDoux, 2005). The amygdala is the initial detector of the stressor and activates many systems, including the sympathetic autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenocortical (HPA) axis (Ulrich-Lai & Herman, 2009). ANS activation provokes rapid elevations in for example heart rate and blood pressure, which can be easily measured in an experimental setting (Ulrich-Lai & Herman, 2009). Activation of the HPA axis is classically measured by elevation of glucocorticoid (primarily cortisol) levels (de Kloet, Joëls, & Holsboer, 2005). The hippocampus plays an important role in the regulation of cortisol secretion and emotional memory. Cortical regions such as the anterior cingulate cortex, orbitofrontal cortex and other parts of the prefrontal cortex are thought to provide negative feedback on subcortical structures and thereby regulate the stress response (Gold et al., 2015).

the regulation and mediation of autonomic responses to emotive stimuli (M. L. Phillips, Drevets, Rauch, & Lane, 2003) (fig. 1.1). A decline in amygdala function could therefore lead to decreased aversive reaction. The cognitive control hypothesis (Mather & Carstensen, 2005) on the other hand argues that the positivity effect is a result of older adults' greater focus on regulating emotion. Affective states and autonomic responses to emotional stimuli are regulated by the prefrontal cortex with a reciprocal functional relationship between the amygdala and the prefrontal cortex (M. L. Phillips et al., 2003).

In sum, aging appears to be a period with heightened vulnerability to stress. At the same time healthy aging is associated with positive psychological features. The neural correlates of this paradox are thus far unclear. Importantly, the potential role of acute stress in this phenomenon still remains to be investigated.

Individual differences

Interactions between individual differences in genetic constitution and stress from the environment have been a major research topic since the seminal work by Caspi and colleagues (Caspi et al., 2003). In this study, the authors showed in a large sample that risk allele carriers of the serotonin transporter polymorphism (5-HTTLPR) were more likely to suffer from depression or even attempt to commit suicide, but only when they had also encountered stressful life experiences. Their results were confirmed in a more recent meta-analysis (Karg, Burmeister, Shedden, & Sen, 2011). In addition, underlying neural mechanisms of stress sensitivity were identified in risk allele carriers, such as enhanced activation of the hippocampus and amygdala (Drabant et al., 2012; S. E. Murphy et al., 2012). Since the Caspi study, several other important genetic variations were identified that interact with stress on a neural (Cousijn et al., 2010; Gerritsen et al., 2011) and a behavioral (Amin et al., 2012; van Oostrom et al., 2012) level. This line of research has demonstrated that the interplay between genetic variance and stressful life

events plays an important role in neural and behavioral development, and therefore in the etiological pathways to psychiatric disease (Kendler, 2013).

Interestingly, epigenetic mechanisms (processes that regulate gene expression, such as methylation) may serve as an interface between genes and traumatic life experiences (Zannas & Chrousos, 2017). For example, altered methylation of the promotor region of the serotonin transporter gene polymorphism was found to be associated with unresolved trauma (van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010) and hippocampal gray matter volume (Dannlowski et al., 2014). Evidence suggests that increasing levels of cumulative life stress or higher numbers of stressors induce stronger effects on the methylome. In addition, the epigenome is continually changing during the life cycle and may be particularly susceptible during periods of rapid epigenetic remodeling or periods of inadequate functioning of epigenetic maintenance systems, such as in early development or at advanced age, contributing to the 'windows of vulnerability' to stress described in the previous paragraph (McEwen et al., 2015; Zannas & Chrousos, 2017).

Importantly, progressive knowledge over the years has provided the insight that in most circumstances multiple genes are involved in causing variance in brain structure and function, and similarly multiple brain regions interact when leading to different behavioral phenotypes (Franke, Neale, & Faraone, 2009). One can therefore investigate the consequences of gene-environment interactions at different levels of the endophenotype, such as brain anatomy, brain function, personality traits or psychiatric illness (fig. 1.2).

Personality is an interesting and relevant intermediate phenotype, because of its strong and close association with some psychiatric disorders, as well as its shared genetic risk factors with mental disease (Kendler, Gatz, Gardner, & Pedersen, 2006). The 'Big Five' personality traits are part of a commonly used model to describe personality dimensions. These traits are defined as openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism (McCrae & Costa, 1999). Neuroticism has been found to be one of the most important psychological vulnerability factors for affective disorders (Kotov, Gamez, Schmidt, & Watson, 2010).

1.2 Types of stressors: the role of the environment

Another reason for differences in the consequences of stress is that not every stressor is alike. A little brother taking away your favorite toy or getting stuck in a traffic jam is very different compared to childhood experiences with emotional or physical abuse. Hence, severity, quantity and the duration of the stressor are obvious important factors

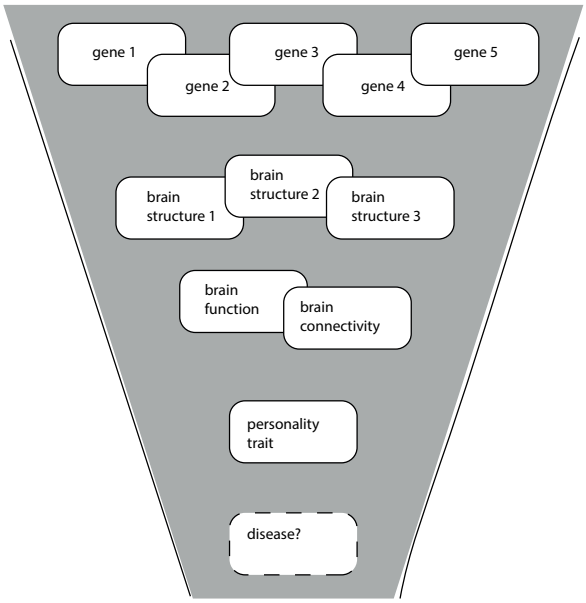


Figure 1.2 Basic model of the endophenotype concept in psychiatry.
Simple but often multiple genetic variations cause differences in brain anatomy, which leads to differences in brain function. Adapted from (Franke et al., 2009).

to take into account. Acute and chronic stress interact with the intensity and duration of the stressor, following an inverted U-shaped curve from acute traumatic effects on neural functioning, through adaptive plasticity during chronic stress to permanent damage after prolonged stress (McEwen et al., 2015).

More recently it has been shown that the type of stressor can also have a distinct impact on the neural and behavioral consequences of stress, with sometimes opposite effects of childhood experiences with deprivation versus more directly threatening experiences such as abuse (McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). More specifically, while deprivation has been associated with underdevelopment of brain regions associated with sensory processing, early exposure to threatening situations is associated with increased activity of emotion processing regions in the brain, representing increased vigilance (McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). Correspondingly, for sexual abuse very specific effects were found in brain regions involved in sensory functions of the female genital area (Heim, Mayberg, Mletzko, Nemeroff, & Pruessner, 2013). Adverse childhood experiences can thus be measured in dimensions of deprivation and threat, and these dimensions are important with respect to their neural and behavioral correlates

(McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). The nature of the stressor therefore seems to be an additional important facet of stress that has often been overlooked in the past.

1.3 Methods to study stress

Understanding the individual differences in stress vulnerability and resilience is crucial for gaining better insight into the mechanisms leading to stress-related psychopathology. Essentially, there are two types of methods that can be used to study the effects of stress in humans. One possibility is to document real-life stressors in people's lives and link this information to cross-sectional data obtained in the laboratory. This information can provide insight into the associations between stress and later measurements, such as brain anatomy, physiological measurements or data obtained with cognitive tasks. This can be done either retrospectively or prospectively. Another way of studying stress is to administer an experimental stressor in a laboratory setting and prospectively assess its effect on parameters such as cognitive measures, affect ratings, bodily stress responses, and brain activity. Here, these methods and the associated effects on the (neural) stress response will be discussed.

Methods using real–life stressors

Real-life stress is most often measured with retrospective methods. Questionnaires can be administered to healthy subjects or patients to obtain relevant information about experiences in the past.

A frequently used questionnaire to assess adverse childhood experiences is the Childhood Trauma Questionnaire (CTQ), which scores traumatic events distributed over five different subscales (Bernstein et al., 2003). While this questionnaire provides a well-standardized overview of experiences of abuse and deprivation in childhood, it does not assess any other high-impact experiences in childhood, such as parental divorce. In addition, events that occurred after childhood are not included. Another example of a frequently used questionnaire is The List of Threatening Life Events (Brugha, Bebbington, Tennant, & Hurry, 1985), which asks participants whether they have experienced a list of predefined events, ranging from moving house to sexual abuse. Participants can indicate whether they experienced these events before or after the age of 16 years, or even in the past year. This questionnaire includes a wider range of moderate to severe life stressors and also includes events later in life.

In the light of investigating the consequences of stress, a major advantage of these retrospective methods is that they assess stressors using a naturalistic approach: the

stressors are real-life stressors that have not been manipulated by researchers and really occurred in the everyday life of the participants. One important disadvantage of these methods is the risk of selectively reporting events from the past. For example, a patient with a depressed mood will more easily recall negative events from the past than a healthy person (negative memory bias) (Beck, 2008). In addition, since there is no randomized experimental manipulation, no causal inference can be made between the adverse events and later obtained data. Nonetheless, in stress research these methods are still very useful, mainly because of the ethical difficulties of experimentally inducing severe or even traumatic stress in participants.

One exception here is the prospective Bucharest Early Intervention Project (Zeanah et al., 2003). In this study, institutionalized children in Romania were at very young age randomized to foster care or institutionalization, and compared to a control group of never institutionalized children. Since foster care did not exist before and was only available in a limited number of families, this study was considered to meet ethical requirements and has provided a large amount of relevant causal information on the consequences of early life stress (Teicher, Samson, Anderson, & Ohashi, 2016). For example, responses of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis to psychosocial stress were remarkably blunted in 12-year-old institutionalized children compared with controls (McLaughlin et al., 2015). In the Netherlands the TRAILS study has prospectively followed a large cohort of adolescents and monitored their responses to stress for the last 15 years, showing for example that abnormal recovery of the stress-induced cortisol response can predict later stress-related psychopathology (Nederhof et al., 2015; Ormel et al., 2012).

Information about participants' life stressful events can consequently be associated with experimental data, such as brain anatomy, brain function, brain connectivity, and general medical disorders as well as major psychiatric disorders (Nemeroff, 2016). For example, there is strong evidence that childhood maltreatment is associated with structural deficits in the adult hippocampus, corpus callosum, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and dorsolateral prefrontal cortex (PFC), whereas functional deficits are consistently found in the amygdala (Teicher et al., 2016) (fig. 1.1). Brain connectivity also seems altered after childhood adversity, with decreased centrality of emotion regulation regions and decreased connectivity of the default mode network (Teicher et al., 2016; Teicher, Anderson, Ohashi, & Polcari, 2013). Finally, childhood adversity has been associated with many different psychiatric disorders such as anxiety disorders, depression and disruptive disorders (Nemeroff, 2016; Oldehinkel & Ormel, 2015), but also psychosis (Misiak et al., 2017) and bipolar disorder (Palmier-Claus, Berry, Bucci, Mansell, & Varese, 2016). Mechanisms through which ad-

verse childhood experience may lead to these brain alterations and disorders include changes in neuroendocrine, neuroimmune and neurotransmitter systems (Nemeroff, 2016; Nusslock & Miller, 2016).

Inducing stress in the laboratory

Stress effects can also be prospectively studied by the administration of an acute experimental stressor in a laboratory setting. The most commonly used laboratory task that is developed induce psychosocial stress is the Trier Social Stress Task (TSST) (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014; Kirschbaum, Pirke, & Hellhammer, 1993). During this standardized task, a brief preparation period is followed by a test period during which the subject is required to deliver a free speech concerning their suitability for employment in a mock job interview and to perform mental arithmetic in front of an audience that is trained to withhold verbal and non-verbal feedback. This task is found to reliably elevate biomarkers of psychosocial stress (Foley & Kirschbaum, 2010). Another method is the Cold Pressure Test (CPT), originally developed to induce blood pressure elevation for hypertension studies, during which participants are instructed to keep their hand in ice cold water for as long as possible (Lovallo, 1975). This test also has a socially evaluated version, with videotaping and close monitoring by a researcher with lack of empathy (Schwabe, Haddad, & Schachinger, 2007). The Maastricht Acute Stress Test combines features of the TSST and the CPT and has also been found to strongly elevate stress responses (Smeets et al., 2012).

An important disadvantage of these tasks is the limited possibilities of performing the task in neuroimaging settings, for example in the confined space of a MRI scanner. To circumvent this problem, several adapted paradigms were developed more recently. One example is the Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005). This test consists of a series of computerized mental arithmetic challenges, along with social evaluative threat components that are built into the program or presented by the investigator. Another stress induction paradigm adapted for neuroimaging settings was based on experiments using negative mood induction, which is most often done with salient movie clips (Kreibig, 2010). A similar approach using highly aversive movie material was developed to induce stress (Qin, Hermans, van Marle, Luo, & Fernández, 2009). Showing these movie clips to participants has been found to elicit a mild physiological stress response in an efficient and well-controlled manner (Cousijn et al., 2010; Hermans et al., 2011; van Marle, Hermans, Qin, & Fernández, 2009).

Notably, the stress that is induced with these prospective methods is always acute stress and relatively mild, since inducing severe and long-lasting forms of stress would inevitably lead to ethical problems. The same holds for the artificial nature of the stress:

everyday life stressors in the participants own environment would be very difficult to experimentally create. Nevertheless, the laboratory environment is still very important to prospectively study stress effects, since it allows for a well-controlled setting to investigate behavioral, physiological and neural responses to well-defined stressors.

1.4 Summary and open questions

The response to threat or a stressor is an adaptive response which may ultimately lead to maladaptation, and therefore in the pathophysiology of psychiatric disease. Stress interacts with many factors, such as age, sex and personality traits when conveying this risk. In addition, the characteristics of the stressor itself moderate these effects. Psychological stress is therefore an important topic of research, and several experimental paradigms have been developed to investigate its effects. However, more research is needed to gain better understanding of the complexity of moderating factors involved in the consequences of stress in healthy individuals.

In this thesis we set out to address the following outstanding questions:

Q1 What are the influences of genetic variance and sex on the neural effects of childhood stress?
Q2 What is the influence of type of stressor when investigating the effects of childhood stress on the brain?
Q3 What is the influence of personality traits such as neuroticism on the neural stress response?
Q4 How is the neural stress response affected by healthy aging?

Outline of this thesis

Here, the experimental approaches that were used to address these questions will briefly be introduced. Part 1 addresses questions 1 and 2, whereas in part 2 questions 3 and 4 are discussed.

In Part 1 the effects of childhood stress on brain structure (box 1.2) in young adults is discussed. In our first experiment (**chapter 2**) we investigated the moderating effect of sex on gene-environment interaction effects on the volume of the hippocampus. We used questionnaires to assess a history of childhood adversity in a large sample of young, healthy adults who were genotyped for the serotonin transporter gene polymorphism (5-HTTLPR), a polymorphism that has been associated with depression.

In the second experiment (**chapter 3**), the same questionnaire was used in a large sample of young, healthy adults, however here we focused on gray matter differences associated with different types of childhood adversity. The potentially moderating factor of sex was taken into account as well.

In Part 2 the effects of acute stress on brain function in young and older adults is discussed. In these two experiments we used the experimental design depicted in fig. 1.3. In the first experiment (**chapter 4**) we investigated how trait neuroticism correlated with stress-related differences in neural responses to emotional facial expressions. This experiment was done in a large sample of young, healthy males. During the sec-

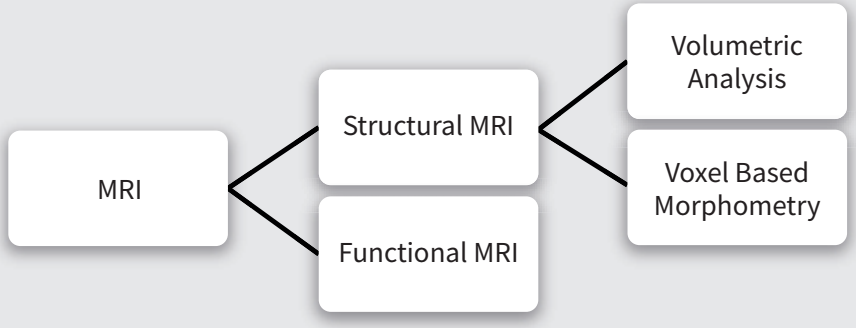
Box 1.2

Brief overview of the neuroimaging methods used in this thesis

Magnetic Resonance Imaging (MRI) is a commonly used technique that can be used to image the human brain. MRI makes use of differences in magnetic properties of different tissues within the brain, giving contrast to an MR image. One important advantage of MRI over other brain imaging techniques is its relatively good spatial resolution.

Structural MRI provides information on the *anatomy* of the brain. Volumetric analyses can be used when investigating whether a certain a priori defined brain region is larger in patients than control subjects, for example. In addition to information about volumes of well described brain regions, more explorative methods can also be used to compare the anatomy between different populations, or across time. Voxel Based Morphometry (VBM) is a more recently developed method which compares gray or white matter concentrations per voxel in the brain (Ashburner & Friston, 2000).

Functional MRI (fMRI) involves the measurement and analysis of brain function. To this end, the magnetic properties of the blood-oxygen level dependent (BOLD) response are used to estimate neuronal activity (Huettel et al., 2004). When the BOLD-response is compared between different experimental conditions, this contrast can provide information about condition-specific changes in activity in different parts of the brain.



ond experiment (**chapter 5**) we used the same paradigm in older males (60-75 years old) compared to young males matched for trait anxiety and educational levels. This allowed us to investigate age-related differences in physiological and neural stress responses.

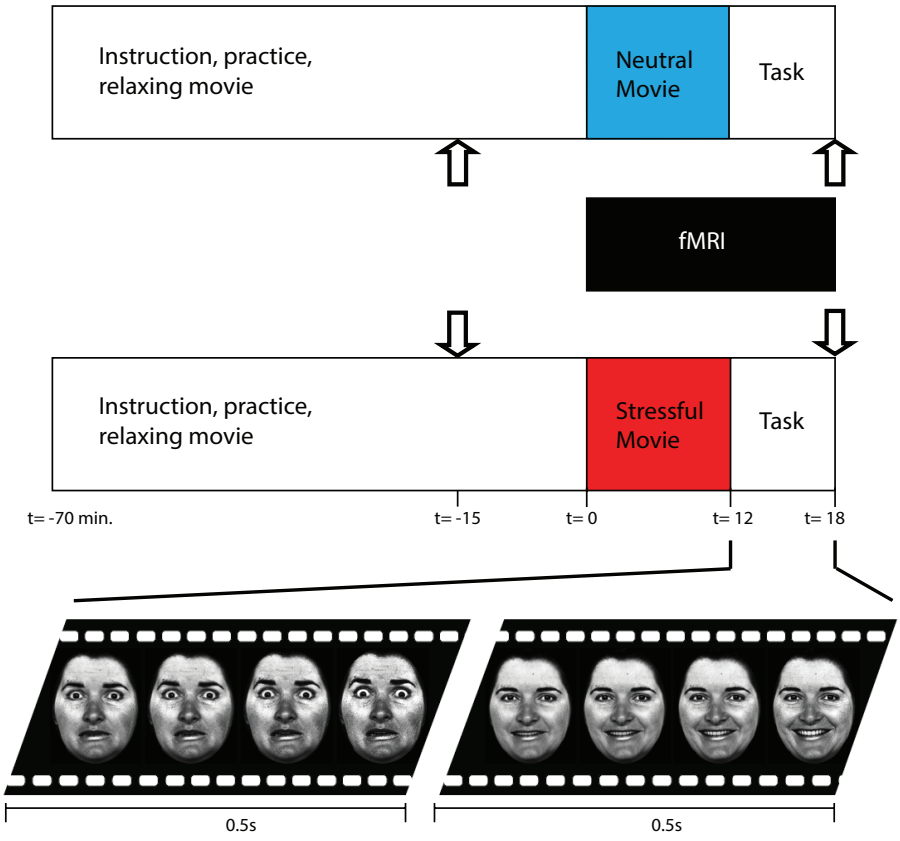


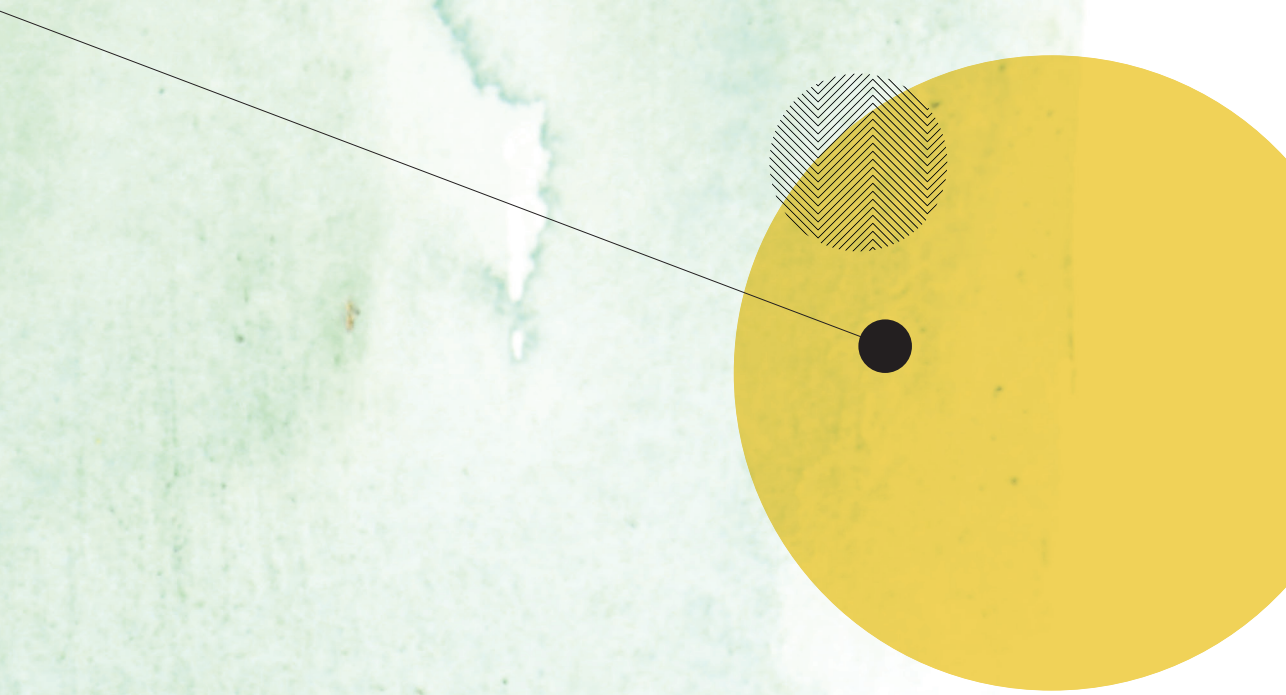
Figure 1.3 Overview of the experimental design used in chapters 4 and 5.
Time is indicated in minutes relative to the start of the movie. Subjects entered the scanner after a period of relaxation, after which either the stressful movie or the neutral movie followed. An emotional face processing task was administered in the two conditions. Physiological stress parameters were measured just before entering the scanner (t = − 15) and after the task (t = 18), indicated by the arrows.

PART 1

THE EFFECTS OF CHILDHOOD STRESS ON BRAIN STRUCTURE

CHAPTER 2

SEX MODULATES THE INTERACTIVE EFFECT OF THE SEROTONIN TRANSPORTER GENE POLYMORPHISM AND CHILDHOOD ADVERSITY ON HIPPOCAMPAL VOLUME



Abstract

The common genetic variation of the serotonin transporter-linked polymorphic region (5-HTTLPR) has been related to depressive symptoms, in particular after stressful life events. Although it has been investigated in the past, results suggesting that the 5-HTTLPR genotype also affects hippocampal volume are often inconsistent and it remains unclear to what extent reduced hippocampal volume is influenced by the effect of stressful life events and 5-HTTLPR genotype. Moreover, sex, which is known to affect the prevalence of depression substantially, has not been taken into account when trying to disentangle the interactive effect of common genetic variation and environmental stressors on the hippocampus. We investigated this potentially relevant three-way interaction using an automatic MRI-based segmentation of the hippocampus in 357 healthy individuals. We determined the 5-HTTLPR genotype as a biallelic locus and childhood adversity (CA) using a standard questionnaire. An interaction for hippocampal volume was found between the factors sex, genotype, and severe CA (p=0.010) as well as an interaction between genotype and severe CA (p=0.007) in men only. *Post hoc* tests revealed that only male S'-allele carriers with severe CA had smaller hippocampi (p=0.002). Interestingly, there was no main effect of genotype in men, while female S'-allele carriers had smaller hippocampi than L'L'-carriers (p=0.023). Our results indicate that sex modulates the interactive effect 5-HTTLPR genotype and CA have on hippocampal volume. While the S'-allele is associated with hippocampal volume independent of CA in women, men only have smaller hippocampi if they carry the risk allele and experienced severe CA.

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2.1 Introduction

Depression is a highly disabling and recurrent disease. Despite intensive research optimal treatment is still lacking and hence, primary prevention of the disorder is a major goal. Therefore, recent research has focused on understanding vulnerability factors for depression investigating in particular the effect of common genetic variations and environmental factors on brain regions involved in the pathophysiology of depression. Understanding the effect of genetic variation in serotonin neurotransmission is one important focus of this line of research after an initial study has shown that short (S) allele carriers of the serotonin transporter linked polymorphic region (5-HTTLPR) exhibit more depressive symptoms, when they had also experienced stressful life events and childhood adversity (Caspi et al., 2003). After two meta-analyses failing to replicate this result (Munafò, Durrant, Lewis, & Flint, 2009; Risch et al., 2009), a recent and more complete meta-analysis (Karg et al., 2011) has confirmed that 5-HTTLPR moderates indeed the relationship between stress and depression. Moreover, a strong association between the S-allele and increased stress sensitivity was found in particular when the authors included only those studies that used childhood maltreatment as their stress measure (Karg et al., 2011). Childhood maltreatment is known to cause HPA-axis dysregulation and extensive neurobiological alterations (Heim & Nemeroff, 2001) and can therefore be considered to be a form of severe childhood adversity.

It is important to identify brain regions that are sensitive to gene by environment interactions (Brown & Hariri, 2006). Here the hippocampus has gained attention because reduced hippocampal volume is related to both childhood psychosocial stress (Tottenham & Sheridan, 2009) and depression (Sapolsky, 2000; Videbech & Ravnkilde, 2004). Moreover, small hippocampal volumes are associated with poor clinical outcome, a mechanism through which potential gene by environment interactions further impact on the course of the disease (MacQueen & Frodl, 2010). The hippocampus has a dense serotonergic innervation (Jacobs & Azmitia, 1992) so that common genetic variation of serotonergic neurotransmission could affect hippocampal morphology. Interestingly, developmental imaging studies using spectroscopy found a significantly lower hippocampal N-acetylaspartate concentration indexing altered neurogenesis, in S-allele as compared to homozygous L-carriers (Gallinat et al., 2005).

While Frodl and colleagues showed that healthy subjects homozygous for the S'-allele exhibit smaller hippocampi compared to L/L carriers (Frodl et al., 2008), (Canli et al., 2006) only found evidence for a greater hippocampus in L/L carriers related to greater life stress (see also (J. Cole et al., 2011; Dutt et al., 2009; O'Hara et al., 2007)). Along the continuum to psychopathology, depressed patients carrying the S'-allele and a history for emotional childhood neglect had smaller hippocampal volumes compared

to patients carrying the risk S'-allele without childhood adversity as well as compared to patients with childhood adversity, but homozygous for the non –risk allele (Frodl et al., 2010).

Besides the relatively small sample sizes of previous genetic neuroimaging studies investigating this interaction, gender differences may have potentially confounded the results. We hypothesize that sex has a modulatory effect on this gene by environment interaction on hippocampal volume. Sex steroids are potent modulators of brain development and the hippocampus contains sex steroid receptors (MacLusky, Clark, Naftolin, & Goldman-Rakic, 1987). Moreover, there is evidence that stress hormone responses are different between men and women (Kudielka & Kirschbaum, 2005). The hippocampus is a core structure regulating stress hormone release and its morphology itself is also affected by stress, suggesting that sex-specific mechanisms could moderate the gene by environment interaction with respect to hippocampal volume (Lupien et al., 2009).

Most relevant with respect to understanding the vulnerability factors of depression, sexual dimorphism is not only present in the neural development of the hippocampus. (Heim, Shugart, Craighead, & Nemeroff, 2010) proposed that gene by environment interactions might occur in a sex-specific manner as an explanation for the higher prevalence of depression in women. Thus far, only studies using clinical outcome measures support the effect gender has on the association of 5-HTTLPR and stressful life events in the prevalence of depression (Brummett et al., 2007; Eley et al., 2004; Sjöberg et al., 2005), bypassing possible modulating actions of the brain.

To test the complex interaction between sex, genotype, and CA in affecting hippocampal volume, one needs a large sample of subjects, most optimally a cohort unaffected by consequences of disease and treatment. Investigating young, healthy subjects avoids potential effects of (chronic) disease and medication and thus, it allows a more direct, unconfounded investigation of potential correlates of vulnerability. Therefore, we set out to investigate 357 young, healthy subjects using automatic volumetry of the hippocampus based on 3Tesla MRI, genotyping and a questionnaire on CA.

2.2 Methods and materials

Participants

A total of 357 (136 male, 221 female) subjects were included in this study. All participants gave written informed consent and the study was approved by the local ethics

committee (CMO Region Arnhem-Nijmegen, the Netherlands). The sample consisted of healthy subjects of Caucasian descent, with a mean (SD) age of 24.3 (6.3) years for males and 23.3 (5.1) years for females. They were screened before participation in this study by self-report questionnaires: participants were excluded if they had a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, medication (except hormonal contraceptives) or illicit drug use during the past 6 months, history of substance abuse, current or past alcohol dependence, pregnancy, lactation, menopause or magnetic resonance imaging contraindications.

Genotyping

Genetic analyses were carried out at the Department of Human Genetics of the Radboud University Nijmegen Medical Centre, in a laboratory which has a quality certification according to CCKL criteria. High molecular weight DNA was isolated from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada) according to the protocol supplied by the manufacturer. The 5-HTTLPR genotype was the genotype at the single nucleotide polymorphism (rs25531) in this region, with alleles designated as LG, LA, and S (Parsey et al., 2002). Because it is thought that the LG is comparable to the S-allele with regard to gene transcription and function, we reclassified the alleles on the basis of the resulting expression levels of the SLC6A4/5-HTT gene into L'L', S'L' and S'S' genotypes (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). Testing for Hardy-Weinberg equilibrium did not show deviation from the expected distribution ($p>0.05$).

MRI acquisition

Anatomical T1-weighted MRI data were acquired at the Donders Centre for Cognitive Neuroimaging. All scans covered the entire brain and had a voxel size of 1x1x1 mm3.

To make use of the best possible signal to noise ration all images were acquired at 3 Tesla Siemens Trio or TimTrio scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (TR 2300 ms, TI 1100 ms, TE 3.93 ms, 192 sagittal slices, field of view 256 mm). These variations included TR/TI/TE/slices of 2300/1100/3.03/192, 2300/1100/2.92/192, 2300/1100/2.96/192, 2300/1100/2.99/192, 1940/1100/3.93/176, 1960/1100/4.58/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2.

Image data processing

Whole brain segmentation of gray matter, white matter and cerebrospinal fluid was performed using the VBM 5.1 toolbox version 1.19 (dbm.neuro.uni-jena.de/vbm/) in SPM5 (www.fil.ion.ucl.ac.uk/spm/) using priors (default settings). Total volume of gray matter and white matter was calculated by adding the resulting tissue probabilities.

Total brain volume was defined as the sum of white matter and gray matter volume. For the automatic segmentation of the hippocampus we used FIRST v1.2 (www.fmrib.ox.ac.uk/fsl/first/index.html) in FSL 4.1.4 (http://www.fmrib.ox.ac.uk/fsl) (Patenaude, Smith, Kennedy, & Jenkinson, 2011). This method is based on Bayesian statistical models of shape and appearance for seventeen structures from 317 manually labeled T1-weighted MR images. To fit the models, the probability of the shape given the observed intensities is used (Patenaude et al., 2011). In addition, to model intensity at the structural boundary, automatic boundary correction was applied. After segmentation, the volume of the left and right hippocampus was calculated by multiplying the number of voxels in a specific structure with the voxel volume (1 mm^3). To detect obvious segmentation errors (like brain structures located outside the brain) visual inspection of the segmented structures projected onto the T1-weighted MRI scans was done using the software MRIcroN Version Beta 7 (www.mricro.com/mricron). Total hippocampal volume was defined as the sum of left and right hippocampal volumes.

Assessment of childhood adversity

Childhood adversity (CA) was assessed using an adapted version of the List of Threatening Life Events developed by Brugha and colleagues with the aim of assessing the stress of life events (Brugha et al., 1985). This inventory encompasses life events that are likely to occur relatively frequently and score relatively high on long-term threat. Participants were asked whether they had experienced a list of predefined events before the age of 16. This list comprised events like death of a parent, severe illness of self or spouse, moving house and abuse. Presence of CA was defined as having experienced at least one CA before the age of 16 years.

Negative mood

The Positive Affect Negative Affect Schedule (PANAS) consists of two 10-item scales designed to provide brief measures of positive and negative affect (Watson, Clark, & Tellegen, 1988). The two scales index two distinct, but moderately negatively correlated, factors. PANAS was used as a covariate in our analysis in order to correct for possible recollection bias.

Recollection bias is not only found in depressed subjects, but also in never-depressed individuals that are for example neurotic (Chan, Goodwin, & Harmer, 2007) or family members of MDD patients (Jaenicke et al., 1987). In the light of these findings, it is therefore possible that those of our healthy subjects with more risk factors for depression are also more likely to report negative life events due to recollection bias, resulting in an overestimation of the effect of life events when not correcting for PANAS scores.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18. Significance level was p = 0.05. An interaction effect of sex, genotype and CA was tested by adding a product term (sex*5-HTTLPR*CA) to the fully adjusted models. When significant, these interaction effects would be further explored using two-way interaction models and simple post-hoc T-tests with Bonferroni correction. With Bonferroni correction our post-hoc significance level will be p = 0.05/(2x2x2) = 0.006. Covariates in all analyses were: age, level of education, MRI protocol and negative mood state measured with PANAS. Moreover, we used total brain volume (TBV) as a covariate to control for gender specific differences in brain size that could affect the outcome of our specific volumes.

The main effect of 5-HTTLPR on hippocampal volumes was examined by conducting analyses of covariance (ANCOVA). The main effect of CA on hippocampus was examined in an equivalent analysis. Individual differences between the different genotypes were explored using simple post-hoc T-tests. When comparing 5-HTTLPR genotypes, we also compared S'/S' and S'/L' together to L'/L' (thus S'-allele carriers versus non S'-allele carriers respectively) to investigate whether potential effects are more generally related to the presence of the S'-allele.

2.3 Results

There were no significant differences in covariates between the two sexes, except for total brain volume (larger in males, p<0.001) and educational level (higher in females, p=0.037) (Table 2.1). Note that all analyses were stratified for these aspects.

To make sure that the variation in MRI protocols did not systematically influence our automatic segmentation of the hippocampus, we conducted an ANOVA with MRI protocol as independent and total hippocampus volume as dependent variable. This analysis showed no significant effect of MRI protocol on hippocampus volume ($F(16,340)=20.4$, $p=0.204$). Moreover, the different MRI protocols were equally distributed between the genotypes ($\chi 2=30.5$, df=32, p=0.542), CA groups ($\chi 2=11.9$, df=16, p=0.750) and sexes ($\chi 2=23.7$, df=16, p=0.096).

Three–way interaction between CA, genotype and sex

We did not find an interaction effect of CA with genotype and sex. Since a previous meta-analysis suggests a particularly strong association between the S'-allele and more severe childhood adversity like maltreatment (Karg et al., 2011), we selected the following items from the List of Threatening Life Events: death of a close relative or friend and victim of abuse, war or disaster (items 3, 6, 7, 8, 9, 17 and 18; see Table

Table 2.1 Characteristics of the study population.

	Male (n=136)	Female (n=221)	p-value
Age (SD)	24.3 (6.3)	23.3 (5.1)	0.101
Total brain volume (ml) (SD)	1379.2 (98.4)	1229.7 (97.4)	0.000*
Educational level			0.037*
Low (%)	30 (22.1)	30 (13.6)	
Intermediate (%)	17 (12.5)	29 (13.3)	
High (%)	89 (65.4)	162 (73.3)	
5-HTTLPR			
S'/S' (%)	39 (28.7)	76 (34.4)	0.295
S'/L' (%)	66 (48.5)	88 (39.8)	0.124
L'/L' (%)	31 (22.8)	57 (25.8)	0.613
CA			
≥ 1 event (%)	97 (71.3)	150 (67.9)	0.555
≥ 1 severe event (%)	79 (58.1)	113 (51.1)	0.229
PANAS negative symptoms (SD)	11.9 (3.6)	12.1 (4.1)	0.756

* Indicates a significant difference ($p < 0.05$). Chi-square tests were used for genotype and CA (childhood adversity) differences, all other tests were T-tests.

2.2). These events were marked as severe CA. The selection of these specific events was motivated by evidence that death of a loved one and any violent event or sexual trauma in childhood is highly associated with subsequent PTSD symptoms (Breslau, Chilcoat, & Kessler, 1999; Copeland, Keeler, Angold, & Costello, 2007). Most importantly, with respect to our research question we found that there was a three-way interaction between severe CA, genotype and sex on total hippocampus volume (p=0.01), even when not corrected for the PANAS (p=0.016).

Two–way interaction between severe CA and genotype

When further exploring this interaction by computing a two-way analysis with the factors genotype x severe CA for males and females, separately, we found a significant interaction in males (p=0.007/ p=0.01), but not in females (p=0.638/ p=0.740). When re-analyzing these data for S'-allele carriers versus L'/L' carriers, the three-way interaction sex x genotype x severe CA (p=0.045/ p=0.062) and the two way interactions genotype x severe CA in males (p=0.003/ p=0.005) and in females (p=0.344/ p=0.299) confirmed our results (Table 2.3), indicating that we identified an effect that is more generally related to the presence of the S'-allele. Finally, post-hoc T-tests performed in the male subgroup only revealed that severe CA is associated with smaller hippocampi in male S'-allele carriers (p=0.002/ p=0.002, also significant after Bonferroni correction), but not in males with the L'/L' genotype (p=0.600/ p=0.686) (Fig. 2.1). We also followed-up the two-way interaction between genotype and severe CA by separately investigating

Table 2.2 List of threatening life events before the age of 16. Adapted from Brugha et al. (1985).

1	Serious illness or injury to subject
2	Serious illness or injury to a close relative
3	Death of first-degree relative including child or spouse or death of close family friend or second-degree relative
4	Separation due to marital difficulties
5	Broke off a steady relationship
6	Verbal or physical aggression in family
7	Verbal or physical aggression outside of family
8	Sexual abuse or violence in family or relationship
9	Sexual abuse or violence outside of family or relationship
10	Pregnancy or childbirth
11	Marriage or living together
12	Other family changes (adoption or children moving out of house)
13	Serious financial problems
14	Problems with police
15	Moving house
16	Long term separation from (one of) parents
17	Victim of war violence
18	Witness of disaster or severe accident

the effects of severe CA and genotype on hippocampal volume. We found no main effect of either of these factors in men (maximum p=0.995/p=0.991). While females also showed no main effect of severe CA (p=0.611/ p=0.579), female L'/L' carriers showed larger hippocampi compared to S'-allele carriers (p=0.023/ p=0.024) (Fig. 2.2). This last effect however, is not significant after Bonferonni correction (p>0.006).

2.4 Discussion

In this study we show for the first time that sex modulates the interactive effect of the 5-HTTLPR genotype and childhood adversity on hippocampal volume. We found a significant three-way interaction, which was driven by the fact that only male S'-allele carriers exhibited an association between severe CA and smaller hippocampal volume (Fig. 1). Contrary, in females differences in hippocampal volume were only found as a function of genotype (independent of CA) whereby S'-allele carriers showed smaller hippocampal volumes. Interestingly, we neither found a main effect of genotype nor CA in men. Taken together, our results imply a sex-specific dissociation in genetic and environmental effects on hippocampal volume in a healthy sample that is suggestive of a sex-specific vulnerability to develop depression. A smaller hippocampus, which re-

Table 2.3 Genotype effects on hippocampal volume.

	5-HTTLPR genotype			S'-allele carriers versus L'/L'		
	F	Mean difference (95% CI)	p-value	F	Mean difference (95% CI)	p-value
3-way	6.634	-	0.010*	4.001	-	0.045*
2-way						
Females	0.222	-	0.638	0.897	-	0.344
Males	7.162	-	0.007**	8.553	-	0.003**
Main effect of genotype						
Females	5.599	-	0.061	5.187	-0.205 (-0.0381- -0.029)	0.023*
Males	0.013	-	0.993	0.004	0.010 (-0.286-0.307)	0.947
All	2.786	-	0.248	2.587	-0.127 (-0.283-0.028)	0.108
S'/S' versus L'/L'						
Females	2.843	-0.173 (-0.374- 0.028)	0.092	-	-	-
Males	0.000	0.001 (-0.347- 0.350)	0.995	-	-	-
All	1.355	-0.107 (-0.287- 0.073)	0.244	-	-	-
S'/L' versus L'/L'						
Females	5.465	-0.230 (-0.424- -0.037)	0.019*	-	-	-
Males	0.009	0.015 (-0.300- 0.330)	0.925	-	-	-
All	2.738	-0.142 (-0.311- 0.026)	0.098	-	-	-

In main effects of 5-HTTLPR genotype df=2, in other effects df=1. * Indicates p<0.05, ** indicates p<0.01.

flects a risk factor to develop depression (Amico, 2011) can already be found in females with the more pathogenic allele of 5-HTTLPR, whereas in males it may be only present if both genetic and environmental risk factors co-occur.

Another reason for inconsistencies with previous studies could be found in the use of different segmentation methods for the hippocampus. Many different segmentation protocols for the hippocampus exist and discrepancies are known to occur, most frequently at specific anatomical boundaries such as the anterior hippocampal-amygdala border (Konrad et al., 2009). The deformable model of FSL FIRST has been shown to give accurate and robust results for the segmentation of 15 subcortical structures. For the hippocampus the median quantitative Dice overlap measures are in the range of

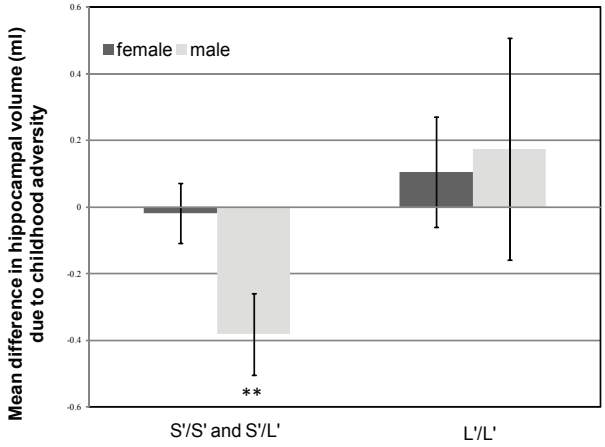

Figure 2.1 Differences in the effect of severe childhood adversity on total hippocampus volumes between subjects with different genotypes.
Results are indicated per sex and corrected for age, level of education, TBV (total brain volume), MRI protocol and PANAS score. This graph shows a significant negative effect of a history of severe CA (childhood adversity) in male S'-allele carriers only. Error bars represent standard errors. ** Indicates p<0.01.

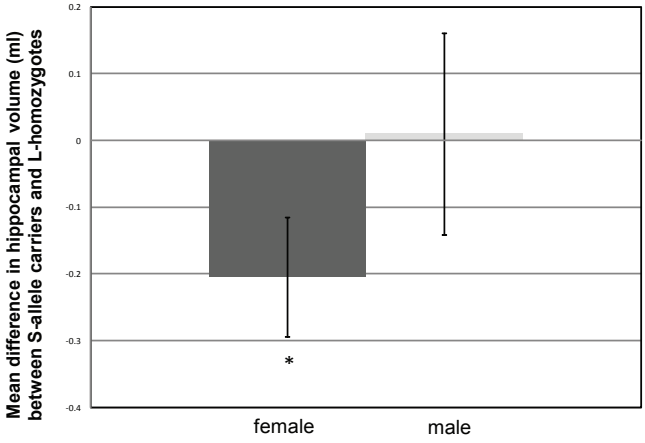

Figure 2.2 Differences in total hippocampus volumes of S'–allele compared to L'/L' carriers per sex when corrected for age, level of education, TBV (total brain volume), MRI protocol and PANAS score.
This graph shows that female S'-allele carriers have a significantly smaller hippocampal volume than female L'/L' carriers. Error bars represent standard errors. * Indicates p<0.05.

0.8 to 0.844, which is comparable to or better than other automated methods (Patenaude et al., 2011).

Our results support the data of (Frodl et al., 2008) showing that in healthy subjects the S'-allele is the risk allele for smaller hippocampal volumes. In the light of missing additional evidence on the hippocampus, in particular (Selvaraj et al., 2011) support a reduction of gray matter in healthy S'-allele carriers in different brain regions. Moreover our findings support the meta-analysis of (Karg et al., 2011) in that we only found an effect of childhood adversity when severe CA was entered in the analysis suggesting that at least in healthy male S'-allele carriers there was no strong impact of milder stressors on hippocampal volume.

The modulatory effect of sex on the interaction between 5-HTTLPR and environmental stressors has thus far only been investigated in clinical studies on depressive symptoms that are not directly comparable to our results. In fact, at first sight those data seem counterintuitive to the data presented here. For example (Eley et al., 2004) showed that an increase in the number of S-alleles increased the likelihood of being in the high depression group only for female adolescents within a high environmental risk group suggesting that the interactive effect may also play a role for females. (Sjöberg et al., 2005) and (Brummett et al., 2007) found also that in women the S-allele, combined with a stressor, was associated with higher depression scores. In males, however, it was the L-allele, combined with a stressor, that was related to more depressive symptoms. It should be noted that all three studies did not reclassify the LG-allele as S'-allele, which could lead to an unbalanced underestimation of the effect per sex (e.g. an underestimation of S'-allele effects in males only if the LG-allele would have been more prevalent in males than females). In addition, two of the three studies investigated adolescents. In adolescence, however, neural sex differences show dynamic changes during development, with a 1-2 years earlier peak in cortical and subcortical gray matter trajectories in females than in males (Lenroot & Giedd, 2010). More specifically, the hippocampus, containing sex steroid receptors, also shows sexual dimorphism in growth rate during this period (MacLusky et al., 1987; Suzuki et al., 2005). Thus, evaluating sex differences in this age can lead to different results than those found in an adult population. Moreover, the interaction between 5-HTTLPR and environmental stressors during development may be related to different psychopathology in the two sexes. The polymorphism has not only been associated with depressive symptoms, but also with other behavioral outcome measures such as social cognition and behavior (Canli & Lesch, 2007). In conclusion it is difficult to compare the effect of the interaction between 5-HTTLPR and environmental stressors on clinical outcome measures with

those on brain correlates that may represent a neurobiological vulnerability in healthy subjects.

The interaction of sex with childhood adversity, possibly due to the neuroprotective effects of estrogen (Sherwin, 2003), may be an important bias when investigating genotype effects on the hippocampus. Estrogen positively modulates serotonergic effects of the brain (Kugaya et al., 2003) and may act as a "brake" on the HPA-axis (Young & Korszun, 2010). In the light of these findings it is not surprising that salivary cortisol increases in response to stress in men are up to twice as high as in women (Kudielka, Hellhammer, & Wüst, 2009). Hence, neurotoxic effects of cortisol, caused by childhood stress, could have a larger impact in males than in females prior to menopause (Sapolsky, 2000). This has already been demonstrated in children with PTSD, where boys show more adverse effects of childhood maltreatment on brain development than girls (De Bellis & Keshavan, 2003). Estrogen can also directly influence hippocampal development by blocking the neurodegenerative effects of glucocorticoids (Pruessner et al., 2010).

Given that the S-allele seems to be associated with an impaired serotonin reuptake activity in the brain (Heils et al., 1996), this may also dampen the modulatory effects of estrogen. Such a direct association between female-specific effects of the S-allele and brain structure is also supported by the main effect of the S-allele on hippocampal volume in females in our data.

A major strength of our study is the large sample size of healthy men and women. One limitation is the use of questionnaires in the assessment of childhood adversity. However, by making the distinction between CA and severe CA, we expect to have created a more specific assessment of childhood adversity. Finally, by using the PANAS as a covariate in our analysis, we have corrected for recollection bias, in which negative mood would influence memory by making it more sensitive to recall negative events.

A clear limitation is that we could not replicate our data in an independent sample which should be the main goal of future studies. It is possible that we underestimate the interactive effect of the aforementioned factors because we may have excluded those subjects who were more affected by CA and developed neuropsychiatric disorders with hippocampal damage, such as PTSD (Karl et al., 2006). In addition, it is possible that our population is too young to show the full extent of CA effects on the hippocampus and that the effects may have been even larger when using an older population (Tottenham & Sheridan, 2009). Future studies should address these issues ideally comparing subjects of different age groups throughout adulthood. Finally, it

is important to mention that our study population does not seem to suffer from less life events than the general population (Cuijpers et al., 2011), but at the same time does not suffer from mental health problems due to the exclusion criteria applied. This implies that our sample consists of subjects belonging to the more resilient part of the population and therefore adequately reflects the healthy segment of a putative health-disease continuum.

In summary, our results show that sex significantly modulates the effects 5-HTTLPR genotype and childhood events have on hippocampal morphology. While in women the S'-allele alone is sufficient to reduce hippocampal volume, in men a history of severe CA modulates the effect within S'-allele carriers. Even though these results require replication, our findings contribute to the understanding of sex differences in the pathophysiology of depression and indicate a mechanistic account of how a specific risk allele and adverse events may increase the vulnerability for depression. Future studies aiming to investigate the effects of the serotonin transporter linked polymorphic region should consider the sex-specific nature of this gene by environment interaction, thereby reducing the chance of false negative results.

CHAPTER 3
CHILDHOOD ABUSE AND DEPRIVATION ARE ASSOCIATED WITH DISTINCT SEX-DEPENDENT DIFFERENCES IN BRAIN MORPHOLOGY

Abstract

Childhood adversity (CA) has been associated with long-term structural brain alterations and an increased risk for psychiatric disorders. Evidence is emerging that subtypes of CA, varying in the dimensions of threat and deprivation, lead to distinct neural and behavioral outcomes. However, these specific associations have yet to be established without potential confounders such as psychopathology. Moreover, differences in neural development and psychopathology necessitate the exploration of sexual dimorphism. Young healthy adult subjects were selected based on history of childhood adversity from a large database to assess gray matter (GM) differences associated with specific subtypes of adversity. We compared voxel-based morphometry data of subjects reporting specific childhood exposure to abuse (n=127) or deprivation (n=126) and a similar sized group of controls (n=129) without reported childhood adversity. Subjects were matched on age, gender and educational level. Differences between CA-subtypes were found in the fusiform gyrus and middle occipital gyrus, where subjects with a history of deprivation showed reduced GM compared to subjects with a history of abuse. An interaction between sex and CA-subtype was found. Women showed less GM in the visual posterior precuneal region after both subtypes of CA than controls. Men had less GM in the postcentral gyrus after childhood deprivation compared to abuse. Our results suggest that even in a healthy population CA subtypes are related to specific alterations in brain structure, which are modulated by sex. These findings may help understand neurodevelopmental consequences related to childhood adversity.

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3.1 Introduction

Childhood adversities (CA) are among the most consistently documented risk factors for psychiatric disorders (Cuijpers et al., 2011; McLaughlin et al., 2012). They are in particular linked to affective, addiction, and personality disorders and possibly associated with distinct phenotypes within these disorders (Gilbert et al., 2009; Heim et al., 2010; McLaughlin et al., 2012). To learn more about the effects of these adversities on brain structure, it is essential to study healthy individuals where the consequences of CA are not confounded by the possible effects of psychopathology (Dannlowski et al., 2012). Indeed such studies have highlighted numerous long-term structural differences in the brain related to CA (Dannlowski et al., 2012; Lim, Radua, & Rubia, 2014; Lupien et al., 2009). Most consistently, changes in gray matter (GM) volume in the prefrontal cortex, sensory association cortices, anterior cingulate gyrus, the amygdala, hippocampus, insula, striatum, and the cerebellum have been found in healthy individuals when compared to controls with no history of adverse childhood experiences (Dannlowski et al., 2012; Edmiston et al., 2011).

Recently, it has been suggested that different types of adverse childhood experiences may lead to distinct morphological alterations in the brain, possibly corresponding to different types of psychopathology in adulthood (Humphreys & Zeanah, 2014; McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). For example, cortical thinning in the somatosensory cortex representing the female genital area was found exclusively in women that had experienced sexual abuse compared to other forms of childhood trauma (Heim et al., 2013). In subjects with a specific history of emotional abuse, thinning in areas involved in emotion processing such as the medial prefrontal cortex (mPFC) and precuneus have been found (Heim et al., 2013; van Harmelen et al., 2010). Additionally, it was proposed that a distinction could also be made between adversity in the form of deprivation, such as absence of the expected social input, and direct threat, such as in active abuse. These two types of adversity potentially lead to different (neural) outcomes (Edmiston et al., 2011; Humphreys & Zeanah, 2014; McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). Deprivation could lead to changes in the association cortex, involved in higher cognitive and social processes, whereas abuse could give rise to alterations in circuits involved in emotional learning (McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). Although these approaches seem promising, practical problems such as co-existing psychopathology and the frequent co-occurrence of different subtypes of adversity have complicated the understanding of their specific impact on neural development.

Our first aim was therefore to assess healthy subjects for specific regional gray matter volume differences corresponding to different types of childhood adversity. To this end, we selected subjects from a database of more than 2700 subjects by their reported history of specific childhood events. We then created two matched groups that had experienced items from either the 'abuse' or 'deprivation' categories. Because of the healthy nature of our population, we also included relatively mild indicators of deprivation, such as death of a close relative (e.g. caring grandparent).

We hypothesized that subjects with a history of deprivation would specifically show GM reductions compared with a matched control group in somatosensory brain regions and association cortex (McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014). Subjects with a history of abuse were hypothesized to differ from the controls especially in brain regions involved in emotion processing and emotional learning, such as the amygdala and hippocampus, ventromedial and dorsolateral prefrontal cortex, and the precuneus (Heim et al., 2013; McLaughlin, Sheridan, & Lambert, 2014a; Sheridan & McLaughlin, 2014; van Harmelen et al., 2010).

An important secondary question was to find out whether the two distinct types of childhood adversity were associated with different GM correlates in the two sexes. Girls and boys have been found to show distinct regional developmental trajectories of gray matter during childhood and adolescence (Lenroot & Giedd, 2010; Rijpkema et al., 2011). The occurrence of adverse events during these periods could therefore have gender-dependent associations with GM in specific brain regions (Tottenham & Sheridan, 2009). Moreover, the type of event could also have a different impact. Importantly, men and women not only differ in prevalence of affective disorders, but also in their etiological pathways to depression (Kendler & Gardner, 2014). For example, men seem more vulnerable to (sexual) abuse, life events and instrumental problems, while for women problems relating to interpersonal warmth and relationships have greater impact (Kendler & Gardner, 2014). Finally, men have often been considerably underrepresented in the majority of studies looking into childhood events, making it difficult to generalize these results to both sexes, while the prevalence of CA in men is substantial (Edwards, Holden, Felitti, & Anda, 2003). We therefore hypothesized that sex could interact with the type of childhood adversity in its effect on GM structure.

3.2 Methods and materials

Participants

The study was part of the Cognomics Initiative's Brain Imaging Genetics (BIG) project at the Donders Institute for Brain, Cognition and Behavior of the Radboud University in Nijmegen, the Netherlands (www.cognomics.nl). Participants were screened before participation in this study by self-report questionnaires. They were excluded if they had a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, medication (except hormonal contraceptives) or illicit drug use during the past 6 months, history of substance abuse, current or past alcohol dependence, pregnancy, lactation, menopause, or MRI contraindications. A total of 2737 subjects was included in the BIG project database at the time of our analysis.

Subjects were specifically selected for the current analysis based on their history of childhood adversities (CA). An age limit (age 18 to 35 years) was set to homogenize the groups. Of note, this is also the age range of onset for most forms of CA-related psychopathology (Lupien et al., 2009). Specific types of adversities were assessed using an adapted version of the 'List of Threatening Life Events' (Brugha et al., 1985). Participants were asked whether they had experienced a list of predefined events (a) before the age of 16 years and/ or (b) at or after the age of 16 years (S3.1). Three groups were based on CA-type: an 'abuse group', a 'deprivation group', and a control group. Subjects were assigned to the abuse group if (i) they reported any verbal, physical and/ or sexual abuse before the age of 16 years and (ii) did not report any items indicating deprivation before 16 years. These deprivation items consisted of a history of separation from parent, severe financial problems, health problems of a close relative and/ or death of a close relative. The latter two are not explicitly mentioned in common definitions of childhood deprivation (e.g. (Sheridan & McLaughlin, 2014)), however are considered here as indicators that a child was deprived of expected social input (e.g. death of a close relative that most likely resulted in the absence of a caregiver). Importantly, we only included subjects who indicated a history of separation from parent under 16 years, since separation from parent later in life does not necessarily indicate deprivation (e.g. leaving home to study abroad). Subjects who reported any items indicating deprivation before the age of 16 years and did not report any lifetime abuse were assigned to the deprivation group. Subjects in the control group did not report any items before age 16 or any lifetime abuse.

The abuse group was the smallest group (n=131) in our sample. Therefore, subjects from the two other groups were matched to the abuse group based on age, sex, and educational level in order to obtain equal group sizes. This gave us a subset of 393 sub-

jects (131 per group) for our study. After the exclusion of 11 subjects due to insufficient data quality for VBM analysis or missing data, we had a final sample of 382 subjects (167 men and 215 women) for this analysis (Table 3.1). The mean (SD) age was 22.1 (3.7) years for men and 22.0 (3.4) years for women.

Affect rating

The negative affect scale of the Positive Affect and Negative Affect Schedule (PANAS) was used to assess negative affect at the time of filling in the life event questionnaire (Watson et al., 1988). Negative affect was entered as a covariate in our analysis to cor-

Table 3.1 Characteristics of the study population.

	Abuse group N=127	Deprivation group N=126	Control group N=129	p-value
Age (years)				
Range	18-34	18-35	18-35	
Mean (SD)	22.4 (4.0)	21.7 (3.0)	22.1 (3.4)	NS
Male %	46	41	44	NS
Adversity before the age of 16 years*				
Verbal or physical aggression in family (%)	76 (60)	0	0	
Verbal or physical aggression outside of family (%)	55 (43)	0	0	
Sexual abuse or violence in family or relationship (%)	12 (9)	0	0	
Sexual abuse or violence outside of family or relationship (%)	13 (10)	0	0	
Serious illness or injury to a close relative (%)	0	94 (75)	0	
Death of close relative (%)	0	99 (79)	0	
Long-term separation from (one of) parents (%)	0	46 (37)	0	
Severe financial problems (%)	0	5 (4)	0	
Adversity after the age of 16 years				
Verbal or physical aggression in family (%)	30 (24)	0	0	
Verbal or physical aggression outside of family (%)	17 (13)	0	0	
Sexual abuse or violence in family or relationship (%)	3 (2)	0	0	
Sexual abuse or violence outside of family or relationship (%)	5 (4)	0	0	
Educational level				NS
Low (%)	21 (17)	19 (15)	20 (16)	
Intermediate (%)	19 (15)	18 (14)	19 (15)	
High (%)	87 (69)	89 (71)	90 (70)	
Mean PANAS negative items (SD)	13.8 (4.5)	13.6 (4.5)	13.8 (4.7)	NS
Total brain volume (ml) (SD)	1255.4 (118.7)	1244.1 (137.8)	1240.3 (107.9)	NS
MRI field strength				NS
1.5T (%)	63 (50)	59 (47)	60 (47)	
3T (%)	64 (50)	67 (53)	69 (53)	

*Percentages add up to >100% because one subject can score multiple items

rect for possible recollection bias due to current affective state. When not correcting for PANAS scores, the effect of life events could be overestimated, since those individuals with currently lower mood may also be more likely to report negative life events (Chan et al., 2007). We also conducted our main analysis without PANAS correction, which enabled us to verify whether this covariate influenced our findings.

MRI acquisition

Anatomical T1-weighted MRI data were acquired at the Donders Centre for Cognitive Neuroimaging. All scans covered the entire brain and had a voxel size of $1 \times 1 \times 1 mm^3$.

For 48% (n=182) of the subjects images were acquired at 1.5 Tesla Siemens Sonata or Avanto scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (Repetition Time (TR) 2300 ms, Inversion Time (TI) 1100 ms, Echo Time (TE) 3.03 ms, 192 sagittal slices, field of view 256 mm). These variations included a TR/TI/TE/slices of 2730/1000/2.95/176, 2250/850/2.95/176, 2250/850/3.93/176, 2250/850/3.68/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2.

For all other subjects images were acquired at 3 Tesla Siemens Trio, TimTrio, or Skyra scanners (Siemens, Erlangen, Germany), using small variations to a standard T1-weighted 3D MPRAGE sequence (TR 2300ms, TI 1100ms, TE 3.93ms, 192 sagittal slices, field of view 256mm). These variations included TR/TI/TE/slices of 2300/1100/3.03/192, 2300/1100/2.92/192, 2300/1100/2.96/192, 2300/1100/2.99/192, 1940/1100/3.93/176, and 1960/1100/4.58/176, and the use of GRAPPA parallel imaging with an acceleration factor of 2.

Image data processing

Local differences in gray and white matter volume related to group differences were studied using a voxel-based morphometry approach (Ashburner & Friston, 2000). For this analysis, T1-images were processed with the default procedures and routines of the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm/) within SPM8 (http://www.fil.ion.ucl.ac.uk/spm/). Using a unified model, T1-images were bias-field corrected, segmented into gray matter, white matter, and cerebro-spinal fluid, and registered with the standard MNI152 brain template by way of high-dimensional DARTEL warping (Ashburner, 2007). The resulting images were modulated by the non-linear part of their DARTEL warp field and smoothed with a 10 mm FWHM Gaussian smoothing kernel, providing for an analysis of relative differences in regional gray and white matter volume, corrected for individual brain size. A detailed description of the protocol can be found in the VBM8 manual (http://dbm.neuro.uni-jena.de/vbm8/VBM8-Manual.pdf).

Data analysis

For the main analyses, group was entered as a factor and age, sex, MR scanner field strength, and PANAS negative scale were entered as covariates. These covariates were added to ensure that small differences between our matched groups would not influence our findings. For the interaction analyses, an additional product term (group*sex) was added to the fully adjusted model. When significant, the interaction effects were further explored by constructing a new model per sex. Group was then entered as a factor and age, scanner, and PANAS negative scale served as covariates.

All statistical tests were family-wise error rate (FWE) corrected for multiple comparisons across the entire brain ($p_{FWE}<0.05$) or across all voxels in a region of interest (ROI) using a small volume correction (SVC; $p_{SVC}<0.05$). Small volume corrections were only applied in the follow-up analysis, when the interaction analysis returned clusters that were significant on a whole brain level (initial $P_{FWE}<0.05$). ROIs were based on a standardized neuroanatomical atlas and used for post-hoc small volume correction (Tzourio-Mazoyer et al., 2002). Mean beta values of significant clusters were extracted for visualization purposes only, using the SPM toolbox MarsBar, version 0.42 (http://marsbar.sourceforge.net).

All other statistical analyses were performed using PASW Statistics 19. Significance level was p=0.05. Comparisons between the three groups were made using one-way ANOVAs. When comparing men to women or when directly comparing two CA groups, independent t-tests and chi-square tests were used for respectively continuous and categorical variables.

3.3 Results

The matched groups did not differ with respect to mean age, sex, educational level, negative affect scores, total brain volume, and MRI field strength (Table 3.1). In addition, with the exception of total brain volume (larger in males), there were no significant sex differences for these variables. We found no indications for group differences in TR (χ^2=3.08, df=6, p=0.80) and TE (χ^2=24.05, df=18, p=0.15). Also, there were no significant group * gender interaction effects in TR (F=1.855, df =1, p=0.174) and TE (F=0.015, df =1, p=0.904). Finally, there were no significant group differences within the separate groups of men and women (S3.2A-B).

Specific effects of CA

In an initial analysis across both men and women, no differences in GM volume between either of the childhood adversity groups were found when compared to the control

group. Additionally, we found no significant main effect of childhood adversity (abuse group and deprivation group combined) on GM volume when compared to the control group (whole brain pFWE > 0.05). However, in line with a divergent effect of different CA subtypes, we observed differences between the two CA groups in the left fusiform gyrus and the right middle occipital gyrus, whereby subjects in the deprivation group had smaller gray matter volumes than subjects in the abuse group (whole brain pFWE < 0.05; Figure 3.1, S3.3, S3.4). When repeating this analysis without correction for negative affect, our two clusters were still significant (pFWE < 0.05), suggesting that negative affect did not account for these group differences.

Interactions with sex

Next, we tested the hypothesis that sex interacts with CA effects on GM differences. We found a significant interaction between CA history and sex in the right visual posterior precuneal region (CA groups < control group, p_{FWE}=0.001) (Margulies et al., 2009) (S3.3). Post-hoc analyses indicated that women with a history of CA (independent of type) had smaller GM volume in this region than women in the control group (p_{FWE}=0.006) (Figure 3.2, S3.5A, S3.6A), while in men no significant differences were found.

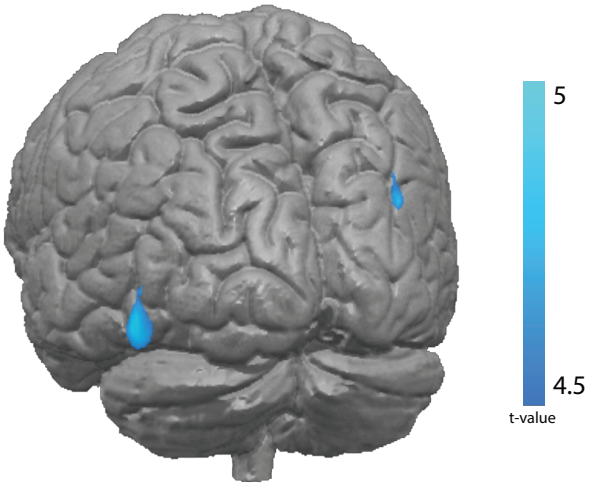


Figure 3.1 Significant decrease in gray matter volume in subjects with a history of deprivation (n=126) versus those with a history of abuse (n=127) in childhood in the fusiform gyrus and middle occipital gyrus.
A 3D rendering is shown with color-coded differences in blue, thresholded at pFWE<0.05 for the t-contrast deprivation group < abuse group.

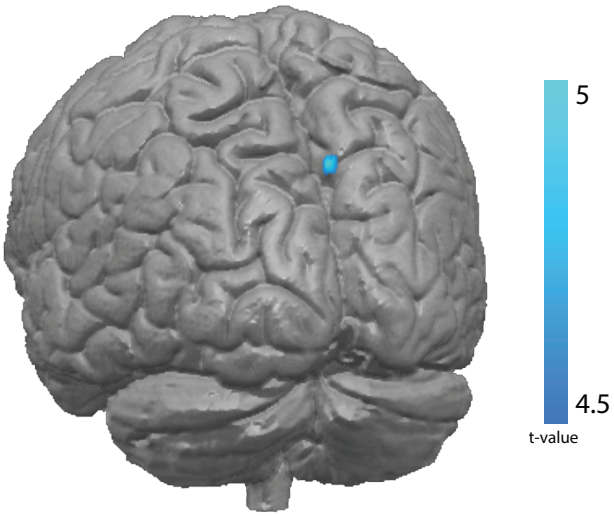


Figure 3.2 Significant decrease in gray matter volume in women with a history of childhood deprivation or abuse (n=143) versus controls (n=72) in the right inferior visual posterior precuneal region.
A 3D rendering is shown with color-coded differences in blue, thresholded at $p_{FWE}<0.05$ for the t-contrast CA groups < control group.

A second significant group*sex interaction was found in the left postcentral gyrus (S3.3). Here, male subjects from the deprivation group had significantly smaller GM volumes in the bilateral postcentral gyrus than men in the abuse group ($p_{SVC}<0.05$) (Figure 3.3, S3.5B, S3.6B).

Correction for later abuse

Since 34% of the subjects in the abuse group also reported having experienced abuse after the age of 16 years, we tested whether our findings could be due to the events that occurred later in life. To this end, we added the binary variable of later abuse (yes or no) as a covariate to our fully adjusted model. Later abuse was defined as any of the items indicating 'abuse' in Table 3.1 occurring after age 16 years. After this correction, only the sex- related differences in GM volume in the postcentral gyrus (men with deprivation versus men with abuse) were no longer detectable. All other findings remained significant (see for all data S3.7).

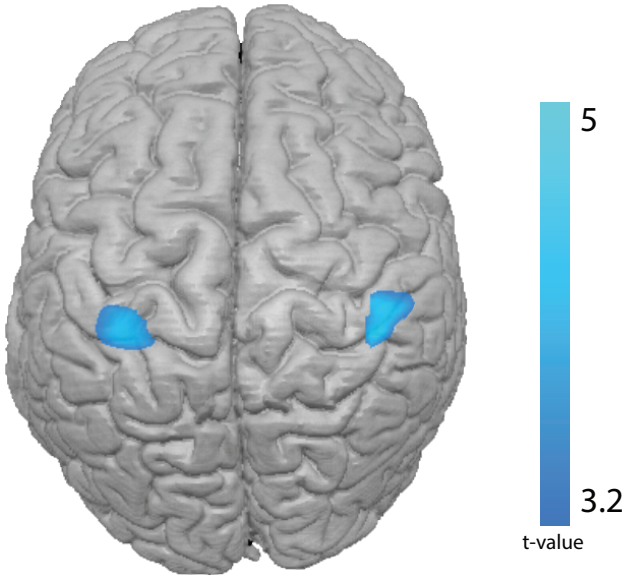


Figure 3.3 Significant decrease in gray matter volume in men with a history of childhood deprivation (n=52) versus men with a history of abuse (n=58) in the postcentral gyrus.
A 3D rendering is shown with color-coded differences in blue, for the t-contrast deprivation group < abuse group thresholded at $p_{uncorrected}<0.001$ and masked with an anatomical mask for the bilateral postcentral gyrus.

Comparison of volumes of interest

Surprisingly, we did not find any effects on hippocampus and amygdala volume using our voxel-wise, brain-wide analysis, despite previous evidence of CA-related psychopathology involving these regions (e.g. (Hanson et al., 2015)). Therefore, we decided to revisit these null findings by conducting a volumetric analysis using an automated segmentation technique (S3.8). Here we replicated our previous finding that there were no differences in bilateral hippocampus or amygdala volume between the three groups (p>0.1) (S3.9).

3.4 Discussion

The present data suggest that even in a healthy sample, subtle CA-specific alterations in GM structure can be found. In line with the hypothesis of specific effects of childhood adversity subtypes, specific associations were found in the fusiform gyrus and middle occipital gyrus, whereby subjects in the deprivation group revealed significantly

smaller GM volumes than those belonging to the abuse group. In addition, sex-specific differences in somatosensory integration areas were found.

The fusiform gyrus and middle occipital gyrus are brain regions responsible for visual processing and multimodal integration (Kravitz, Saleem, Baker, & Mishkin, 2011). More specifically, these areas have been associated with face perception (Kanwisher, McDermott, & Chun, 1997) and scene perception (Dilks, Julian, Paunov, & Kanwisher, 2013), respectively. Enhanced activity in the fusiform gyrus has also been related to the processing of personally familiar faces compared to faces of strangers, which suggests that its function may go beyond simple face perception (Gobbini, Leibenluft, Santiago, & Haxby, 2004). Previous studies reporting structural changes in visual processing areas in relation to childhood adversity are scarce. One study showed reduced cortical thickness in V2 and the left occipital pole after witnessing domestic violence in childhood (Tomoda, Polcari, Anderson, & Teicher, 2012). Another study found that subjects had smaller GM volumes in the fusiform and middle occipital gyrus after sexual abuse in childhood (Tomoda, Navalta, Polcari, Sadato, & Teicher, 2009). While these previous studies all report smaller GM volumes after abuse, our data revealed smaller GM volumes in subjects that had experienced deprivation in childhood. One explanation for this inconsistency could be that, while high-threat environments in humans are often well defined and investigated, the amount of deprivation in these same environments is usually unclear (Sheridan & McLaughlin, 2014), and the contribution of deprivation to the findings in abuse studies is therefore often unknown. Possibly, some of the effects that have been attributed to abuse in the past could be in fact related to deprivation. Notably, from animal models we know that deprivation in early development can have extensive consequences on neural development (Diamond, Lindner, Johnson, Bennett, & Rosenzweig, 1975). Studies in the field of perceptual neuroscience have shown that early visual deprivation in animals and humans leads to radical structural changes, resulting from the reduction of synapses in the primary visual cortex (Leporé et al., 2010; O'Kusky, 1985). In humans, widespread reductions in cortical thickness in regions including the fusiform gyrus and precuneus have been found in Romanian children that had experienced pronounced early-life deprivation, mediating problems with inattention and/ or impulsivity (McLaughlin, Sheridan, Winter, Fox, et al., 2014b; Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012).

Of note, in our subjects experiences of deprivation all occurred within the context of the subject's family, while abuse could also occur outside of the family context, which could suggest a possible bias towards more severe experiences in the deprivation group (Edwards et al., 2003). We tested this hypothesis by dividing the abuse group into two subgroups: one with subjects who reported abuse within the family and one

with subjects who exclusively reported abuse outside their family. Importantly, only a minority (35%) of the subjects in the abuse group exclusively experienced abuse outside their family. Moreover, additional analyses showed that the subjects within the abuse group that had either experienced abuse within or outside their family did not significantly differ from each other in gray matter volume (whole brain pFWE > 0.05), hippocampus or amygdala volume (Supplemental Results). This suggests that the context of the abuse did not significantly influence brain structure in our sample.

We also found two sex-specific effects in somatosensory integration areas. The visual posterior precuneal region was affected in women, both after abuse and deprivation in childhood. This region has been found to have close functional connectivity with the fusiform gyrus and also represents a transition from occipital to limbic connectivity in the precuneus (Margulies et al., 2009). It is therefore a cortical area representing the interplay of sensory input and the processing of emotions. Notably, larger functional connectivity of the precuneus with the hippocampus and ACC in women than men could be one mechanism leading to sex-specific alterations in precuneal GM structure after CA (Zhang & Li, 2012). One other study in women found reduced cortical thickness in this area after experiencing childhood emotional abuse (Heim et al., 2013). In addition, the precuneus has been found to play an important role in the mediation of grief in women (Gündel, O'Connor, Littrell, Fort, & Lane, 2003). Of note, thickness of the precuneal cortex has been shown to be inversely correlated with sensitivity to interpersonal rejection in a large sample of healthy college students, suggesting that this anatomical difference may underlie differences in emotion processing in a healthy population (Sun et al., 2014). The postcentral gyrus was the second region showing sex-specific changes, where males had smaller GM volumes after a history of deprivation than male subjects with a history of abuse. However, since this effect disappeared after correction for more recent abuse, this finding may not be specific for ***childhood*** abuse. In the light of previous findings in a female clinical sample, we had expected to find smaller GM volumes in the somatosensory cortex related to a history of sexual abuse (Heim et al., 2013). While these findings seem contradictory, they in fact highlight the importance of defining sex-specific pathways in the processing of stressful stimuli. Different pathways could be involved in the processing of fearful events in men and women and therefore lead to different neural correlates (Everaerd et al., 2012). For example, in men only, the postcentral gyrus seems to be involved in the processing of fearful faces (Weisenbach et al., 2014), which could be one potential factor of interest in the interaction we found in our sample.

From a developmental perspective, sex differences in neural correlates of childhood adversity may occur because of a number of reasons, such as the influence of gonadal

hormones and different 'sensitive' time windows during neural development in boys and girls (Crozier, Wang, Huettel, & De Bellis, 2014; Lenroot et al., 2007; Young & Korszun, 2010). For example, with respect to our data, a recent study found that connectivity between amygdala sub-regions, the precuneus, and the postcentral gyrus shows an age by sex interaction in adolescents (Alarcón, Cservenka, Rudolph, Fair, & Nagel, 2015). This could be one potential mechanism leading to sex-specific effects in these regions dependent on the timing of adverse events. In psychopathology, these sex differences may contribute to the differences in prevalence of different psychiatric disorders, such as more depression in women and more impulse control disorders in men (Kessler et al., 2006; Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993).

Remarkably, we did not find any differences in gray matter structure of other areas that have often been associated with childhood adversity, such as the amygdala, hippocampus, ACC, and prefrontal cortex (Dannlowski et al., 2012; Edmiston et al., 2011; Hanson et al., 2015). One explanation for this missing finding could be that our population consisted of particularly young, highly educated and healthy subjects with no psychiatric consequences of their early adverse life environments. Consequently, these subjects might be a particularly resilient subset of young adults exposed to childhood stress, and differ in that aspect from patient populations that are traditionally examined for consequences of CA (Lim et al., 2014), or from subjects in longitudinal studies involving severe adversity such as institutionalization (Zeanah et al., 2003). Furthermore, it has been suggested that differences in hippocampal volume after CA are not yet visible in late adolescence (Tottenham & Sheridan, 2009). In addition, we excluded subjects with threatening life events after adolescence, in contrast to most other studies. As a consequence, the differences due to CA within our young, resilient population may be difficult to detect. However, we expect that the differences we were able to detect are particularly robust and representative of consequences of childhood adversity in a healthy population, although our results necessitate replication in different cohorts.

Potentially limiting and maybe related to the healthy nature of our subjects, we found the most pronounced differences between the two CA-groups and not when comparing these groups with a control group. One speculative explanation could be that deprived children are significantly under-stimulated as compared with their peers, which in turn may lead to an underdeveloped visual association cortex (Sheridan & McLaughlin, 2014). In contrast, experience with high threat situations in childhood may increase gray matter volumes in these areas, possibly leading to superior vigilance for fearful and sad facial expression after maltreatment (Leist & Dadds, 2009). Although this reasoning is highly speculative, our findings do stress the importance of specifi-

cally assessing the nature of adverse childhood experiences, and highlight the need for future studies looking into their mechanistic underpinnings.

Importantly, we used a large sample that consisted of healthy subjects without any confounding variables such as somatic or psychiatric disease and with a substantial subset of male participants. In addition, we used a questionnaire that allowed us to correct for possibly confounding contributions of more recent adverse events (Brugha et al., 1985). Limitations are the use of a self-report questionnaire, the absence of information on the exact timing and frequency of the events during development, and the cross-sectional design. In addition, it is possible that not all adverse childhood events were reported by all subjects, because of the sensitive nature of this information.

To conclude, we found partly sex-dependent differences in GM volumes between three well-controlled groups of healthy, young adults that had experienced environments of either abuse or deprivation in childhood and a control group. These differences could potentially give rise to specific changes in mental well-being or symptoms of psychopathology (Edmiston et al., 2011; Hanson et al., 2015). Future studies are needed to expand the current findings by examining behavioral consequences of the observed structural differences in even larger, similarly well-selected populations, such as possible differences in processing of facial expressions or other salient information, differences in parent-child attachment, somatic symptoms in psychiatric disease or somatic disease, all of which have been associated with CA in the past (Cuijpers et al., 2011; Leist & Dadds, 2009; McGoron et al., 2012). Finding specific behavioral consequences of different types of childhood adversity could provide insight into the emergence of distinct neurodevelopmental trajectories, and into potential development of specific psychiatric symptoms and disease in some vulnerable individuals.

3.5 Supplement

S3.1 Adapted list of List of Threatening Life Events.

1	Serious illness or injury to subject
2	*Serious illness or injury to a close relative* [a]
3	*Death of first-degree relative including child or spouse or death of close family friend or second-degree relative* [a]
4	Separation due to marital difficulties
5	Broke off a steady relationship
6	*Verbal or physical aggression in family* [b]
7	*Verbal or physical aggression outside of family* [b]
8	*Sexual abuse or violence in family or relationship* [b]
9	*Sexual abuse or violence outside of family or relationship* [b]
10	Pregnancy or childbirth
11	Marriage or living together
12	Other family changes (adoption or children moving out of house)
13	*Serious financial problems* [a]
14	Problems with police
15	Moving house
16	*Long term separation from (one of) parents* [a]
17	Victim of war violence
18	Witness of disaster or severe accident

a Items indication 'deprivation'
b Items indicating 'abuse'
Adapted from (Brugha et al., 1985). Relevant items are in italics.

S3.2 Characteristics of the study population per sex

(A) Characteristics of the female study population.

	Abuse group N=69	**Deprivation group** N=74	**Control group** N=72	**p-value**
Age (years)				
Range	18-33	18-35	18-34	
Mean (SD)	22.3 (3.8)	21.9 (3.3)	21.9 (3.1)	NS
Adversity before the age of 16 years*				
Verbal or physical aggression in family (%)	45 (65)	0	0	
Verbal or physical aggression outside of family (%)	21 (30)	0	0	
Sexual abuse or violence in family or relationship (%)	8 (12)	0	0	
Sexual abuse or violence outside of family or relationship (%)	9 (13)	0	0	
Serious illness or injury to a close relative (%)	0	60 (81)	0	
Death of close relative (%)	0	62 (84)	0	
Long-term separation from (one of) parents (%)	0	22 (30)	0	
Severe financial problems (%)	0	3 (4)	0	
Adversity after the age of 16 years				
Verbal or physical aggression in family (%)	16 (23)	0	0	
Verbal or physical aggression outside of family (%)	10 (14)	0	0	
Sexual abuse or violence in family or relationship (%)	2 (3)	0	0	
Sexual abuse or violence outside of family or relationship (%)	2 (3)	0	0	
Educational level				NS
Low (%)	9 (13)	11 (15)	8 (11)	
Intermediate (%)	11 (16)	10 (14)	12 (17)	
High (%)	49 (71)	53 (72)	52 (72)	
Mean PANAS negative items (SD)	13.6 (3.6)	13.6 (4.6)	13.9 (4.8)	NS
Total brain volume (ml) (SD)	1184.9 (94.3)	1160.2 (83.3)	1179.2 (82.5)	NS
MRI field strength				NS
1.5T (%)	33 (48)	32 (43)	28 (39)	
3T (%)	36 (52)	42 (57)	44 (61)	

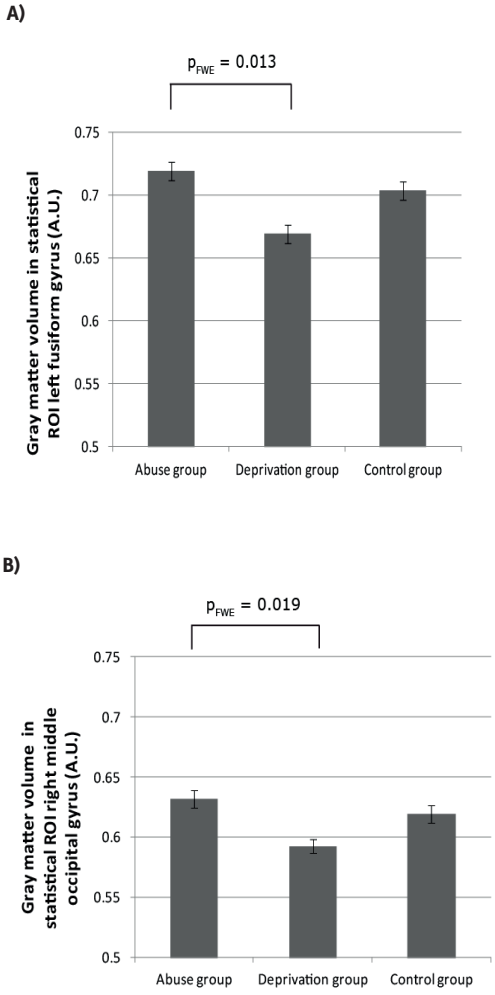
(B) Characteristics of the male study population.

	Abuse group N=58	**Deprivation group** N=52	**Control group** N=57	**p-value**
Age (years)				
Range	18-34	18-30	18-35	
Mean (SD)	22.6 (4.3)	21.4 (2.4)	22.3 (4.7)	NS
Adversity before the age of 16 years*				
Verbal or physical aggression in family (%)	31 (53)	0	0	
Verbal or physical aggression outside of family (%)	34 (59)	0	0	
Sexual abuse or violence in family or relationship (%)	4 (7)	0	0	
Sexual abuse or violence outside of family or relationship (%)	4 (7)	0	0	
Serious illness or injury to a close relative (%)	0	34 (65)	0	
Death of close relative (%)	0	37 (71)	0	
Long-term separation from (one of) parents (%)	0	24 (46)	0	
Severe financial problems (%)	0	2 (4)	0	
Adversity after the age of 16 years				
Verbal or physical aggression in family (%)	14 (24)	0	0	
Verbal or physical aggression outside of family (%)	7 (12)	0	0	
Sexual abuse or violence in family or relationship (%)	1 (2)	0	0	
Sexual abuse or violence outside of family or relationship (%)	3 (5)	0	0	
Educational level				NS
Low (%)	12 (21)	8 (15)	12 (21)	
Intermediate (%)	18 (14)	8 (15)	7 (12)	
High (%)	38 (66)	36 (69)	38 (67)	
Mean PANAS negative items (SD)	14.1 (5.5)	13.6 (4.3)	13.8 (4.7)	NS
Total brain volume (ml) (SD)	1339.1 (85.9)	1361.9 (110.1)	1317.6 (84.2)	0.05
MRI field strength				NS
1.5T (%)	30 (52)	27 (52)	32 (56)	
3T (%)	28 (48)	25 (48)	25 (44)	

S3.3 Gray matter volume differences associated with specific CA.

Contrast	Region	BA	Cluster size	Peak MNI coordinates			Z-score	p value (FWE-corrected)
				X	y	z		
F-test CA (abuse and deprivation) vs control group	NS							
F-test abuse vs control group	NS							
F-test deprivation vs control group	NS							
F-test abuse group vs deprivation group	Inferior temporal gyrus/ fusiform gyrus	37	64	-48	-57	-14	4.59	0.026
	Middle occipital gyrus/ transverse occipital sulcus	19	5	38	-75	25	4.51	0.037
T-test abuse group > deprivation group	Inferior temporal gyrus/ fusiform gyrus	37	201	-48	-57	-14	4.85	0.013
				-44	-51	-3	4.57	0.026
	Middle occipital gyrus/ transverse occipital sulcus	19	34	38	-75	25	4.65	0.019
T-test abuse group < deprivation group	NS							
F-test interaction group (CA vs control group) x sex	Visual posterior precuneal region	7	172	4	-81	42	5.36	0.001
F-test interaction group (abuse group vs control group) x sex	NS							
F-test interaction group (deprivation group vs control group) x sex	Visual posterior precuneal region	7	136	2	-81	40	5.45	<0.001
F-test interaction group (abuse group vs deprivation group) x sex	Postcentral gyrus	2	116	-38	-31	39	4.66	0.019
	Postcentral gyrus	40	3	42	-37	31	4.48	0.042

MNI, Montreal Neurological Institute. CA, childhood adversity (abuse group and deprivation group combined). All analyses are whole brain FWE-corrected.



S3.4 Mean gray matter volumes for the three groups separately

(A) Fusiform gyrus gray matter volume is significantly decreased in the deprivation group compared to the abuse group. Mean gray matter volumes are depicted for the three groups separately, extracted from the significant cluster depicted in figure 1 for visualization purposes. Error bars represent the standard error of the mean.

(B) Middle occipital gyrus gray matter volume is significantly decreased in the deprivation group compared to the abuse group. Mean gray matter volumes are depicted for the three groups separately, extracted from the significant cluster depicted in Figure 1 for visualization purposes. Error bars represent the standard error of the mean.

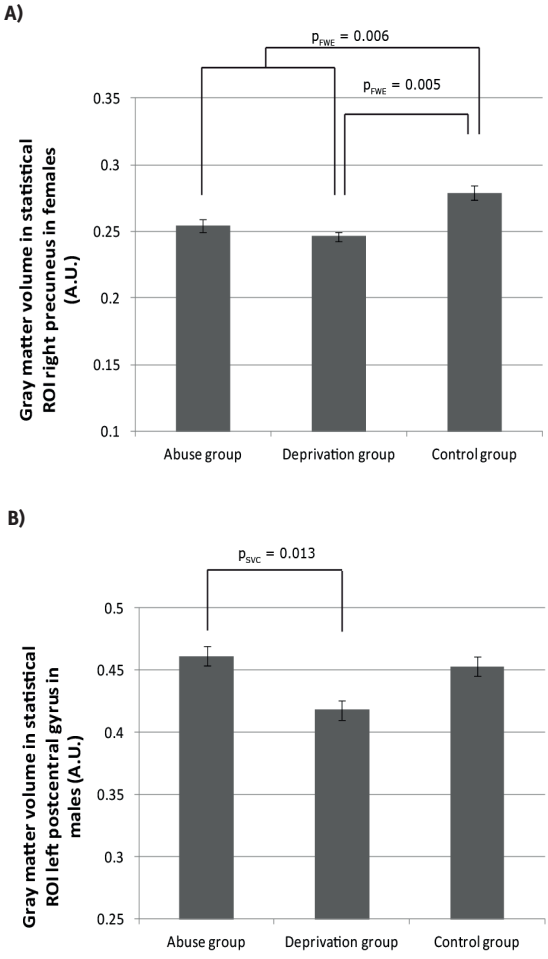
S3.5 Gray matter volume differences associated with specific CA per sex.
MNI, Montreal Neurological Institute. CA, childhood adversity (abuse group and deprivation group combined). *FWE-corrected; # Small volume corrected for the bilateral postcentral gyrus with initial threshold of p <0.001 uncorrected.

(A) Gray matter volume differences in women.

Contrast	Region	BA	Cluster size	Peak MNI coordinates			Z-score	p value
				x	y	z		
F-test CA (abuse and deprivation) vs control group	Visual posterior precuneal region	7	19	4	-81	45	4.77	0.013*
T-test CA (abuse and deprivation) < control group	Visual posterior precuneal region	7	34	4	-81	45	4.91	0.006*
T-test CA (abuse and deprivation) > control group	NS							
F-test abuse group vs control group	NS							
F-test deprivation group vs control group	Visual posterior precuneal region	7	27	3	-81	42	4.83	0.011*
T-test deprivation group < control group	Visual posterior precuneal region	7	44	3	-81	42	4.96	0.005*
T-test deprivation group > control group	NS							
F-test abuse group vs deprivation group	NS							

(B) Gray matter volume differences in men.

Contrast	Region	BA	Cluster size	Peak MNI coordinates			Z-score	p value
				x	y	z		
F-test CA (abuse and deprivation) vs control group	NS							
F-test abuse group vs control group	NS							
F-test deprivation group vs control group	NS							
F-test abuse group vs deprivation group	Postcentral gyrus	2	156	-36	-36	43	3.88	0.026#
			16	45	-24	31	3.64	0.057#
T-test abuse group < deprivation group	NS							
T-test abuse group > deprivation group	Postcentral gyrus	2	198	-36	-36	43	4.04	0.013#
		40	344	45	-34	33	4.33	0.075*
		40	30	45	-24	31	3.82	0.029#
	Middle occipital gyrus/ fusiform gyrus	37	6	-48	-67	-17	4.51	0.036*



S3.6 Mean gray matter volumes for the three groups separately per sex

(A) Visual posterior precuneal region gray matter volume is significantly decreased in the CA group compared to the control group. Mean gray matter volumes are depicted for women only from the three groups, extracted from the significant cluster depicted in Figure 2 for visualization purposes. P-values represent statistical tests at family-wise error rate (FWE) corrected for multiple comparisons across the entire brain (pFWE<0.05). Error bars represent the standard error of the mean.

(B) Postcentral gyrus gray matter volume is significantly decreased in the deprivation group compared to the abuse group. Mean gray matter volumes are depicted for men only from the three groups, extracted from the significant cluster depicted in Figure 3 for visualization purposes. P-values represent statistical tests at family-wise error rate (FWE) corrected for multiple comparisons across the entire brain (pFWE<0.05). Error bars represent the standard error of the mean.

S3.7 Gray matter volume differences corrected for recent abuse.
MNI, Montreal Neurological Institute. CA, childhood adversity (abuse group and deprivation group combined).

(A) Gray matter volume differences associated with specific CA, corrected for recent abuse. All analyses are FWE-corrected.

Contrast	Region	BA	Cluster size	Peak MNI coordinates			Z-score	p value
				x	y	z		
F-test abuse group vs deprivation group	Inferior temporal gyrus/ fusiform gyrus	37	80	-46	-57	-18	4.67	0.019
T-test abuse group > deprivation group	Inferior temporal gyrus/ fusiform gyrus	37	170	-46	-57	-18	4.81	0.009
	Middle occipital gyrus/ transverse occipital sulcus	19	1	-33	-75	1	4.44	0.044

(B) Gray matter volume differences in females, corrected for recent abuse. *FWE-corrected. # Small volume corrected for the bilateral postcentral gyrus with initial threshold of p <0.001 uncorrected.

Contrast	Region	BA	Cluster size	Peak MNI coordinates			Z-score	p value
				x	y	z		
F-test CA (abuse and deprivation) vs control group	Cuneus	19	34	-26	-91	33	4.72	0.017*
	Visual posterior precuneal region	7	2	3	-81	43	4.48	0.044*
T-test CA (abuse and deprivation) < control group	Cuneus	19	102	-26	-91	33	4.85	0.008*
	Visual posterior precuneal region	7	17	3	-81	43	4.63	0.022*

(C) Gray matter volume differences in males, corrected for recent abuse. *FWE-corrected. # Small volume corrected for the bilateral postcentral gyrus with initial threshold of p <0.001 uncorrected.

Contrast	Region	BA	Cluster size	Peak MNI coordinates			Z-score	p value
				x	y	z		
F-test abuse group vs deprivation group	Middle occipital gyrus/ fusiform gyrus	37	11	-48	-66	-17	4.63	0.026*
T-test abuse group > deprivation group	Middle occipital gyrus/ fusiform gyrus	37	43	-48	-66	-17	4.77	0.013*
	Postcentral gyrus	2	40	-36	-33	40	3.39	0.151#
		40	2	45	-24	31	3.22	0.226#

S3.8 Supplemental methods and materials

Data Analysis - FSL

Automatic segmentation of hippocampus and amygdala was performed using the FIRST module of FSL (First version 1.2 (www.fmrib.ox.ac.uk/fsl/first/index.html) (Patenaude et al., 2011) in FSL version 5.0 (www.fmrib.ox.ac.uk/fsl), developed by the Analysis Group, FMRIB, UK). This method is based on Bayesian statistical models of shape and appearance for bilateral amygdala and hippocampus from 317 manually labelled T1-weighted magnetic resonance images. To fit the models, the probability of the shape given the observed intensities is used. In addition, to model intensity at the structural boundary, automatic boundary correction was used (Smith et al., 2004).

After automatic segmentation, volume was calculated using a script in Matlab 7.2 (MathWorks, USA). In this script the volumes of the regional structures of interest were calculated by multiplying the number of voxels with the voxel volume (1 mm3). We compared bilateral amygdala volume and bilateral hippocampal volume. Total brain volume was estimated as the voxel-wise sum of the gray matter and white matter maps, produced by the segmentation done by SPM8.

Consistent with the VBM analyses, we performed an ANCOVA in which group was entered as a factor and age, sex, total brain volume, scanner field strength, and PANAS negative scale as covariates.

S3.9 Comparison of subcortical volumes of interest.

	Abuse group N=127	Deprivation group N=125 [b]	Control group N=129	p-value
Amygdala volume [a] (ml) (SD)	2.82 (0.48)	2.81 (0.41)	2.83 (0.43)	0.792
Hippocampus volume [a] (ml) (SD)	7.96 (0.79)	8.01 (0.94)	7.82 (0.79)	0.079

[a] Volumes are adjusted for the covariates age, sex, total brain volume, scanner field strength, and PANAS negative scale.

[b] Due to technical segmentation problems one subject in the deprivation group was excluded from this analysis.

S3.10 Supplemental results

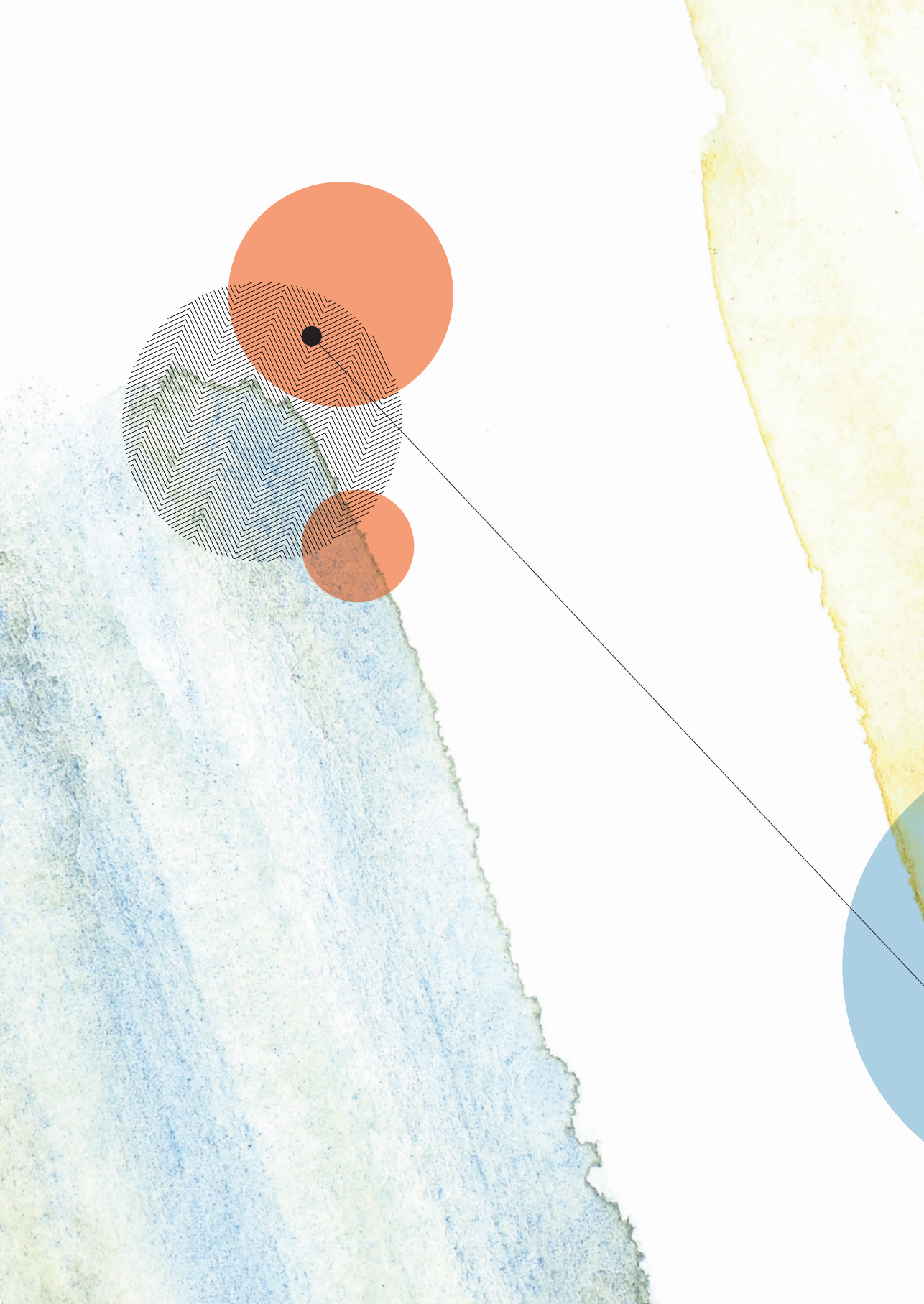
Comparison of subjects with abuse within or outside of family.

To test whether the context of the abusive experience would significantly moderate our findings, we divided the abuse group into subjects that had at least one abusive experience within the family (n=84) and subjects that exclusively reported abusive experiences outside the family (n=47).

We found no significant gray matter volume differences between the subjects who experienced abuse within versus outside their families (whole brain pFWE > 0.05). In addition, these two groups did not differ from each other in terms of age, sex, MR scanner field strength, and PANAS negative scale. Finally, the two groups did not significantly differ in their volumes of the bilateral amygdala and hippocampus, adjusted for the covariates age, sex, total brain volume, scanner field strength, and PANAS negative scale.

PART 2

THE EFFECTS OF ACUTE STRESS ON BRAIN FUNCTION



CHAPTER 4
ASSOCIATION BETWEEN NEUROTICISM AND AMYGDALA RESPONSIVITY EMERGES UNDER STRESSFUL CONDITIONS

Abstract

Increased amygdala reactivity in response to salient stimuli is seen in patients with affective disorders, in healthy subjects at risk for these disorders, and in stressed individuals, making it a prime target for mechanistic studies into the pathophysiology of affective disorders. However, whereas individual differences in neuroticism are thought to modulate the effect of stress on mental health, the mechanistic link between stress, neuroticism and amygdala responsivity is unknown. Thus, we studied the relationship between experimentally induced stress, individual differences in neuroticism, and amygdala responsivity. To this end, fearful and happy faces were presented to a large cohort of young, healthy males (n=120) in two separate functional MRI sessions (stress versus control) in a randomized, controlled cross-over design. We revealed that amygdala reactivity was modulated by an interaction between the factors of stress, neuroticism, and the emotional valence of the facial stimuli. Follow-up analysis showed that neuroticism selectively enhanced amygdala responses to fearful faces in the stress condition. Thus, we show that stress unmasks an association between neuroticism and amygdala responsivity to potentially threatening stimuli. This effect constitutes a possible mechanistic link within the complex pathophysiology of affective disorders, and our novel approach appears suitable for further studies targeting the underlying mechanisms.

Published

Everaerd D., Klumpers F., van Wingen G., Tendolkar I., Fernández G. (2015).

Neuroimage, 112, 218-224.

4.1 Introduction

Major depressive disorder and anxiety disorders are the largest contributors to the worldwide rising burden of mental and behavioral disease, according to a recent report from the World Health Organization (Murray et al., 2012). To investigate the underlying neurobiology of these affective disorders and establish potential targets for treatment, functional neuroimaging studies have examined patients and compared them to healthy controls (Drevets, Price, & Furey, 2008; Etkin & Wager, 2007; Hamilton et al., 2012). One consistent finding is that depressed and anxious patients show stronger amygdala responsivity than controls (Drevets et al., 2008; Etkin & Wager, 2007; Hamilton et al., 2012). This enhanced amygdala responsivity is not a fixed trait, but dependent on the current state of the subject (Delaveau et al., 2011; van Wingen et al., 2011b). For example, a critical precipitating factor for depression is stress, which could potentially be responsible for a shift from vulnerability to maladaptation (Caspi et al., 2003).

Stress can be induced in experimental settings by several different methods and is most often evaluated by changes in heart rate, stress hormones and negative mood (Dedovic et al., 2005; Kirschbaum et al., 1993; Schwabe et al., 2007; van Marle et al., 2009). A state like acute stress, even when mild, triggers a large-scale reallocation of neural processing shifting activity from an executive control network to a salience network including the amygdala, promoting fear and vigilance (Hermans et al., 2011; van Marle et al., 2009). This shift, however, appears to depend on individual trait factors of vulnerability, such as a specific genetic variance or previous exposure to severe stressors (Cousijn et al., 2010; van Wingen, Geuze, Vermetten, & Fernández, 2011a). Thus, to understand the pathophysiology of affective disorders, it is essential to establish the role of these individual differences when examining the effects of acute stress on the brain.

In addition to genetic risk factors, behavioral endophenotypes also cause interindividual variance and represent another step in the pathophysiological pathway to psychiatric disease (Caspi et al., 2003; Franke et al., 2009). One of the most important psychological vulnerability factors for affective disorders is neuroticism (Kotov et al., 2010), a personality trait that by itself is characterized by persistent negative affect or dissatisfaction (Costa & McCrae, 1980; McCrae & Costa, 1999). For example, a longitudinal study has shown that neuroticism increases the risk for a first onset of depression with about 30%, and is therefore considered a strong risk factor (Kendler et al., 2006). Studies that have explored the neural correlates of neuroticism are inconclusive with respect to its neural underpinnings. Specifically amygdala responsivity is sometimes reported to be related to neuroticism, where other studies did not replicate this finding (Canli, 2004; Chan, Norbury, Goodwin, & Harmer, 2009; Kennis, Rademaker, & Geuze,

2013; Servaas, van der Velde, et al., 2013b; Stein, Simmons, Feinstein, & Paulus, 2007). The majority of neuroimaging studies on neuroticism so far, however, did not consider that amygdala responsivity is state dependent. Thus, it is well conceivable that the inconsistencies in the literature about neuroticism and amygdala responsivity might be caused by differences in the subject's state across studies. Indeed, neuroticism has been linked to increased stress responsiveness in physiological studies and heightened stress reactivity has even been suggested to constitute a core element of neuroticism (Depue, 2009; Ormel et al., 2013).

In sum, amygdala responsivity as a functional brain endophenotype can be closely linked to affective disorders, but not consistently to psychological vulnerability factors for these disorders, such as neuroticism. This inconsistency could be due to differences in stress levels between imaging studies probing the association between neuroticism and amygdala responsivity. Therefore, we induced a mild state of acute stress, and a normal control state in a fMRI study design that may allow us to uncover individual differences in amygdala responsivity associated with differences in neuroticism.

4.2 Methods and materials

Participants

We included 120 healthy men (described in table 4.1). Candidates for participation were recruited using a local participant database and advertisements. Screening was conducted by self-report questionnaires before participation. Participants were excluded if they reported a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, medication or illicit drug use during the preceding 6 months, history of substance abuse, current or past alcohol dependence, or MRI contraindications. Women were also excluded because the menstrual cycle is known to influence correlates of the stress response (Fernández et al., 2003; Kirschbaum, Kudielka, Jens, Schommer, & Hellhammer, 1999; Ossewaarde et al., 2011).

One subject was excluded from all analyses because of extreme scores on NEO neuroticism, BDI and STAI-t (> 3 SD above the average sample score). Due to fMRI data artifacts one other participant was excluded, leaving the total sample at 118 subjects. All participants received 60 Euros reimbursement for full participation.

After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the local ethics committee (CMO Region Arnhem-Nijmegen, the Netherlands).

Table 4.1 Characteristics of the study population (n=118).

	Mean (SD)	SD	Range
Mean age during experiment	22.0	2.6	18.1-30.8
Mean NEO-FFI scores			
Neuroticism	28.3*	6.5	14-46
Extraversion	42.6*	6.0	23-54
Openness	40.5*	6.3	28-57
Agreeableness	41.0*	4.1	26-51
Conscientiousness	40.9*	5.2	25-52
Mean STAI-t score	35.4*	7.5	21-58
Mean BDI score	4.3*	4.0	0-17
Mean interval between sessions (days)	12.7	13.0	5-100

*These scores are within the normal range for a young, healthy male population (Creamer, Foran, & Bell, 1995; Hoekstra, Ormel, & Fruyt de, 1996; Knight, 1984). BDI: Beck Depression Inventory. STAI-T: State-Trait Anxiety Inventory (trait form). NEO-FFI: NEO-Five Factor Inventory.

Procedure

All participants took part in a two session study with a randomized, counterbalanced order of the session type (stressful or control) (fig. 1.3). The two sessions were separated by at least five days. The session order was counterbalanced across subjects and not associated with neuroticism scores ($T_{(116)} = 1.404$, $p = 0.163$). On both occasions, participants arrived one hour prior to the MRI session to avoid fluctuations in cortisol levels due to physical activity. During this first hour, participants received information about the study, practiced the tasks they would later have to perform in the scanner, and watched a relaxing nature documentary (Attenborough, 2010). This procedure was extensively standardized in order to create a highly similar experimental setting for all participants. After having watched the documentary, subjects were accompanied to the scanning facility, located in the same laboratory.

To induce a stressful state, highly aversive movie clips were shown in the MRI scanner during one of the sessions (Cousijn et al., 2010; Hermans et al., 2011; van Marle et al., 2009). These clips consisted of scenes of a movie (Noé, 2008) containing extremely aggressive behavior and violence against men and women. As a control condition, neutral, non-arousing scenes of another movie (Fontaine, 2005) were shown in the scanner during a separate session. The stressful and the neutral movie clips were similar in the amount of speech, human (face) presence, luminance, environment, and language. The participants were asked to watch the movie clips from an eye-witness perspective.

Immediately after the movie clip, subjects performed the dynamic facial expression task. This task consisted of passive viewing of photographs of emotionally neutral faces,

morphing into two different emotion types: fearful or happy facial expressions (Ekman & Friesen, 1976). The morphing faces were presented in a block design (three blocks of each emotion, 25s per block, 0.5s per face, avoiding adjacent blocks of the same emotion), interleaved with blocks of fixation cross for baseline reference purposes (three blocks, 25s per block). This task has been found to robustly elicit amygdala activation in previous studies (Cousijn et al., 2010; van Marle et al., 2009).

After this task, the subjects completed several other cognitive tasks in the scanner. These will be reported elsewhere. A structural scan was obtained at the end of the stressful session. The total duration of scanning was approximately 105 minutes per session.

MR data acquisition

MR data of were acquired on a 1.5 T Avanto MR scanner (Siemens, Erlangen, Germany) at the Donders Institute in Nijmegen, the Netherlands. A series of 129 T2*-weighted functional images were acquired using gradient echo-planar imaging (EPI) with the following parameters: 32 oblique transverse slices, voxel size = 3.5 X 3.3 X 3.3 mm, repetition time (TR) = 2.34 s, flip angle α = 90°, echo time (TE) = 35 ms. A 3D magnetization-prepared rapid gradient echo (MPRAGE) anatomical T1-weigthed image was acquired for normalization purposes (176 slices, 1.0 mm isotropic, TR = 2730 ms, TE = 2.95 ms).

Salivary hormone sampling

During each session, three saliva samples were obtained using saliva collection tubes (Sarstedt, Rommelsdorf, Germany). One sample was taken just before the start of the scanning procedure (t=-15s), while the second sample was taken just after the face morphing experiment (t=18s) (fig. 1). Given that diurnal variation in cortisol levels can bias stress-induced cortisol reactions, all testing took place between noon and 6 pm. For reference purposes, participants were asked to collect two extra samples at the same time-of-day on the day before the visits. The average of both samples taken at home was used as baseline for statistical analyses. All samples were stored at -20 °C until assaying.

Laboratory analyses were performed at the Department of Biopsychology, Technical University of Dresden (Dresden, Germany). Biochemical analysis of free cortisol in saliva was performed using a commercially available chemiluminescence immunoassay (IBL Inc.). Concentration of α-amylase in saliva was measured by an enzyme kinetic method (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004).

Psychophysiological measurements

Before scanning (t=-15 min.), resting blood pressure measures of participants were obtained using a standard automatic blood pressure device. Blood pressure was also measured immediately after the task (t=18 min.), using a semi automatic MR-compatible blood pressure device. Heart rate was continuously assessed during scanning by the use of an MR-compatible pulse oximeter.

Questionnaires

Changes in affect during the scanning procedure were assessed using the Positive and Negative Affect Scales (Watson et al., 1988). This was also done at two time points during each test day: at baseline before scanning (t=-15 min.), and immediately after the face processing task (t=18 min.). In addition, participants completed several self-report questionnaires. The following personality/trait scales were used: the Dutch versions of the trait/state anxiety inventory (Spielberger, Gorsuch, & Lushene, 1970), the Beck Depression Inventory (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) and the NEO-FFI (McCrae & Costa, 1999).

Data analysis

MR data quality checks were performed by visual inspection of the structural and functional scans, spike checks, signal-to-noise (SNR) ratio plots and assessment of movement. One subject was excluded based on clear signal drop-out due to scanner malfunctioning. One other subject exceeded our critical movement threshold of 3.5mm (1 voxel) by 0.09mm. We verified the results of this study by performing all analyses without this subject and found no significant differences. Therefore, we decided to keep this subject in our analyses.

Functional MRI data were analyzed using SPM8 (UCL, London). For preprocessing, voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume and were corrected for three-dimensional motion. After realignment and spatial co-registration of both the structural and the functional images, all functional images were normalized into standard stereotactic space (Montreal Neurological Institute [MNI]152 T1-template). Smoothing was performed with a Gaussian kernel of 8 mm full-width at half-maximum. Tissue probability maps were estimated to classify gray matter, white matter and cerebrospinal fluid for each subject and these images were used to create a study specific mean total brain mask (gray + white matter). Subsequently, a General Linear Model was used to characterize voxel-wise signal covariation with task parameters for voxels encompassing the brain mask.

The two emotion types (fearful and happy) were modeled separately as boxcar regressors and convolved with the canonical hemodynamic response function implemented in SPM8. Six realignment parameters were included to model potential movement artifacts. Contrast parameter images were then generated at the single subject level (emotion type compared to fixation). These individual parameter estimate maps were statistically scrutinized at a second level. We used a factorial ANOVA with stress condition and emotion type as within-subject factors, and the individual scores on the NEO-FFI Neuroticism subset of the questionnaire as covariate of interest. This model resulted in statistical parametric maps, which were superimposed upon the mean anatomical image across all subjects for localization purposes. Our statistical threshold for these voxel-wise analyses was set at p=0.05 Family-Wise Error (FWE) corrected for multiple comparisons with Gaussian random field theory as implemented in SPM8.

Given our a priori hypothesis, we then specifically aimed our analysis at the amygdala by applying a small volume correction. For this purpose, we used a predefined anatomical mask of the bilateral amygdala, provided in the Automated Anatomical Labeling (AAL) toolbox in SPM (Tzourio-Mazoyer et al., 2002). Visualizations of correlations were created by superimposing T-contrasts thresholded at $p<0.001$ uncorrected onto the mean anatomical image across all subjects. The MarsBar SPM toolbox (http://marsbar.sourceforge.net) was used to extract the mean responses from individual subjects in post-hoc defined regions of interest (ROIs) for visualization purposes.

All other data (baseline variables, questionnaire scores, heart rate, heart rate variability, blood pressure, cortisol and alpha amylase levels, and PANAS scores) were analyzed in SPSS 19 (IBM) using either dependent or independent T-tests. The stress response was calculated as the difference level between sessions at t=18s (immediately following the dynamic facial expression task). The heart rate was calculated as 60/mean interbeat interval and heart rate variability as the root mean squares of successive differences between successive interbeat intervals. Offline artifact correction and analysis of the heart rate frequency and variability were done with in-house software.

For correlational analyses non-parametric tests were used (Spearman correlations), since extracted amygdala beta values, stress responses, BDI and STAI questionnaires were not normally distributed. All reported analyses were performed with outliers (> 3 standard deviations) removed.

4.3 Results

Study population

NEO, STAI and BDI scores for our population were in a normal range (table 4.1) (Creamer et al., 1995; Hoekstra et al., 1996; Knight, 1984).

Neuroticism scores did not deviate from normality. In line with previous research, neuroticism scores correlated significantly with both STAI-t ($\rho_{(116)}$ = 0.548, p < 0.001) and BDI ($\rho_{(116)}$ = 0.729, p < 0.001) scores and inversely with NEO extraversion scores ($\rho_{(116)}$ = -0.304, p = 0.001).

Stress induction

Stress induction was successful and replicated the results of previous studies using a similar stress induction procedure (Cousijn et al., 2010; Hermans et al., 2011; van Marle et al., 2009).

Salivary cortisol levels were significantly higher following stress induction as compared to the neutral control induction (stress mean: 101.4% of baseline, control mean: 90.9% of baseline, SD=45.7%, $T_{(112)}$ = 1.46, p = 0.016). In this study, no significant effect of stress induction was found on alpha amylase levels (p = 0.865).

Systolic blood pressure and diastolic blood pressure both showed a modest but robust effect of stress induction (respectively stress mean: 108.5 mmHg, control mean: 106.5 mmHg, SD=6.7, $T_{(116)}$ = 3.24, p = 0.002; stress mean: 69.9 mmHg, control mean: 68.4 mmHg, SD=4.7, $T_{(117)}$ = 3.23, p = 0.002). Heart rate increased during the stressful movie (stress mean: 67.1 BPM, control mean: 63.9 BPM, SD=7.6, $T_{(110)}$ = 4.36, p < 0.001), but not significantly during the following dynamic facial expression task (p = 0.374), compared to the movie and task in the control condition. Heart rate variability was decreased during the stressful movie (stress mean: 62.2 ms, control mean: 68.6 ms, SD=26.2, $T_{(109)}$ = 4.36, p = 0.012), but this difference was not significant during the following task (p = 0.157), compared to the movie and task in the control condition.

Negative affect increased substantially after stress induction (stress mean: 17.1, control mean: 13.7, SD=5.9, $T_{(116)}$ = 6.28, p < 0.001) whereas positive affect showed no effect of stress (p=0.943).

In sum, as expected, our measures show that the stressful movie led to significant, but short-lived changes in heart rate, heart rate variability and led to longer lasting increases in blood pressure, cortisol levels and negative affect ratings.

Subsequently, we evaluated possible interactions with trait neuroticism scores. Out of all of the stress measures we assessed, only the relative systolic blood pressure increase correlated with neuroticism ($\rho_{(115)}$ = 0.248, p = 0.007). None of the other measures significantly interacted with neuroticism, suggesting that there was limited influence of neuroticism on physiological or subjectively reported stress in this healthy population.

fMRI results: main effects of task

The presentation of emotional faces produced activation in a distributed network of brain regions (S4.1 and S4.2). These brain regions included the bilateral amygdala, the visual processing network, and prefrontal regions. Across the entire sample, there were no regions that showed stronger activation in the stress as compared to the neutral condition. The response to fearful faces compared to happy faces was greater in the bilateral inferior occipital gyrus and fusiform gyrus (face-processing regions), whereas no regions responded more to happy faces (S4.1). The interaction stress x emotion type revealed no significant clusters. In sum, viewing emotional faces produced highly significant activation in regions important for emotional perception which was slightly stronger for fearful than happy faces. However, there was no significant effect of stress across all our participants. We therefore focused on identifying individual differences in the effects of stress on the emotional processing network.

fMRI results: correlations with neuroticism

Next we examined correlations between neuroticism and brain responses to happy and fearful faces in the stress and control conditions. Whole brain analysis revealed one significant cluster, located in the precentral gyrus (FWE-corrected p<0.01). Given our a priori hypothesis regarding the relationship between emotional face processing and amygdala activation, we then specifically aimed our analysis at the amygdala. As presented in table 4.2, we found an interaction between condition and facial expression in the right amygdala (fig. 4.1a). In the left amygdala a similar pattern was found (peak voxel -22 -4 -26), but only when lowering the statistical threshold to p<0.05 uncorrected.

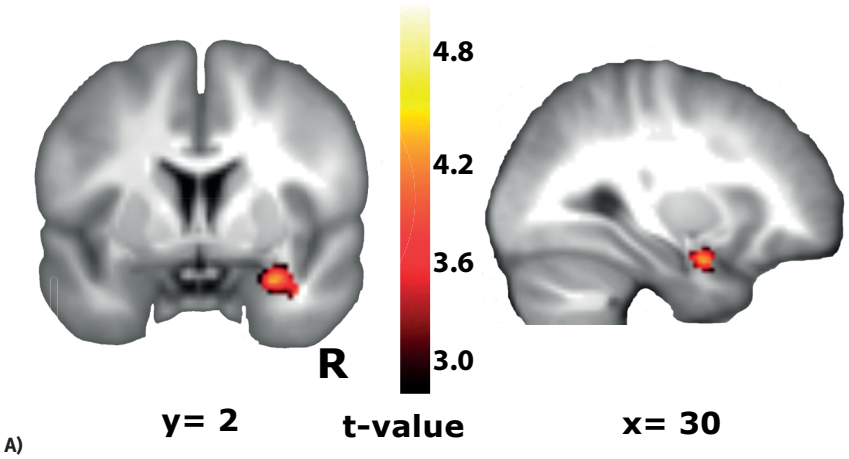
When exploring this interaction, we found that amygdala responsivity was enhanced only for fearful faces in the stressful as compared to the neutral condition for the more neurotic individuals (fig. 4.1b). To avoid inflated correlations (Vul, Harris, Winkielman, & Pashler, 2009), no statistics were performed on these extracted data from the region specified in fig. 4.1a. Importantly, this correlation was also significant for the extracted beta values of the anatomically defined right amygdala (NEO-FFI Neuroticism score versus stress-related signal change for fearful faces: $\rho(116) = 0.186$, p = 0.043).

Table 4.2 Amygdala responsivity in interaction with neuroticism. MNI, Montreal Neurological Institute.

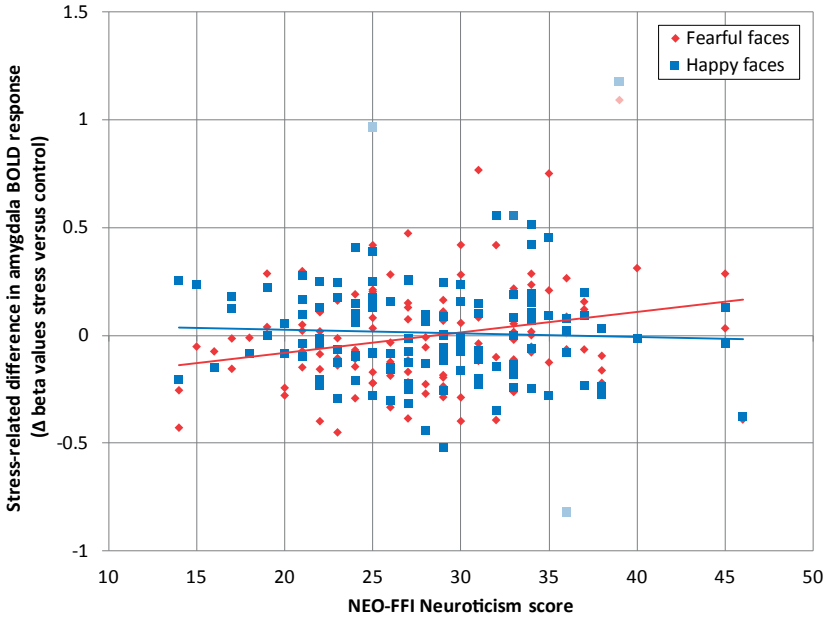
Effect	Region	Hemi-sphere	Cluster size	Peak MNI coordinates			Peak F/T Value
				x	y	z	
F-test interaction stress x emotion x neuroticism	Precentral gyrus	Right	257	32	-8	32	28.02**
	Amygdala	Right	48	30	2	-22	16.99##
Positive interaction stress x emotion x neuroticism *(stress(fear>happy) > neutral(fear>happy))*	Precentral gyrus	Right	340	32	-8	32	5.29**
	Amygdala	Right	74	30	2	-22	4.12##
Negative interaction stress x emotion x neuroticism *(stress(fear>happy) < neutral(fear>happy))*	None	-	-	-	-	-	-
Interaction stress x neuroticism for fearful faces *(stress(fear) > neutral(fear))*	Amygdala	Right	11	30	2	-22	3.47#
Interaction stress x neuroticism for happy faces *(stress(happy) < neutral(happy))*	None	-	-	-	-	-	-

Initial analyses are FWE-corrected: ** p<0.01. All other analyses are small volume corrected with an initial whole brain voxel-wise threshold of $p < .001$ uncorrected for multiple comparisons: # p<0.05, ## p<0.01.

To test whether amygdala responsivity was really only enhanced for the more neurotic individuals, we divided our sample into a low-neuroticism (n=60) and high-neuroticism group (n=58) using a cut-off neuroticism score of 28 (median split). We then made a new factorial ANOVA with stress and emotional facial expression as within-subject factors, and neuroticism (low versus high) as between-subject factor. Testing the interaction stress x emotion (stress(fear>happy) > neutral(fear>happy)) in the high neuroticism group lead to a significant cluster in the right amygdala (small volume corrected, p=0.012, peak voxel 32 -4 -20). Testing the opposite interaction (stress(fear>happy) < neutral(fear>happy) in the low neuroticism group did not lead to any significant effect at the significance level of p<0.001 uncorrected.



A)



B)

Figure 4.1 Positive interaction stress x emotion type x neuroticism in the amygdala.
(A) T-contrasts are projected on an average normalized T1 over all subjects and masked with an anatomical mask for the bilateral amygdala.
(B) Interaction graph with linear trend lines representing the interaction neuroticism x stress for fearful and happy faces separately. The signal change represents the mean activity in the ROI presented in (A).

This pattern of results was confirmed when comparing the extracted beta values of the anatomically defined right amygdala. One-sample t-tests revealed that only for the highly neurotic individuals there was a trend effect for the stress-related signal change for fearful faces ($T(57) = 1.730$, $p = 0.089$ in the high-neuroticism group versus $T(59) = -1.298$, $p = 0.199$ in the low-neuroticism group).

We also followed up on the cluster that was located in the precentral gyrus by exploring the interaction neuroticism x condition per facial expression, but this revealed no significant clusters (FWE-corrected $p > 0.2$).

Importantly, we ruled out that the observed association was a side effect of blood pressure differences between high and low neurotic subjects. In a repeated measures analysis on the extracted amygdala responses, adding blood pressure as a covariate did not significantly change the three-way interaction between stress, neuroticism scores and amygdala responses to the fearful faces (interaction condition x face expression x neuroticism corrected for systolic blood pressure change: $F_{(115, 1)}=14.74$, $p<0.001$).

4.4 Discussion

In the present study we demonstrate for the first time in a large, homogeneous sample of healthy males that higher trait neuroticism levels predict a greater response of the amygdala to fearful faces, but that this effect depends on the current stressful state of the individual. These findings indicate enhanced amygdala responsivity in individuals that are at risk for developing stress-related disorders, yet strongly dependent on the stress level of the individual and the valence of the presented stimulus.

Our results confirm that trait neuroticism is associated with enhanced amygdala responsivity, as has been shown previously by several studies (Chan et al., 2009; Cunningham, Arbuckle, Jahn, Mowrer, & Abduljalil, 2010; Haas, Omura, Constable, & Canli, 2007; Harenski, Kim, & Hamann, 2009), whereas a recent meta-analysis did not find an association between neuroticism and enhanced amygdala response (Servaas, van der Velde, et al., 2013b). The authors speculate that it is either the speed of amygdala recovery (and not the initial response of the amygdala) that is responsible for the negativity bias associated with neuroticism or that reduced connectivity between frontal regions and the amygdala is the basis for heightened emotional responses to negative events. On the basis of our findings, we suggest that some studies did not find any differences in amygdala responsivity because stress levels were uncontrolled. Although prefrontal regions such as the anterior cingulate cortex and ventromedial prefrontal cortex are critical regions for the regulation of amygdala responsivity, the

precise impact of neuroticism on top-down control of these regions over the amygdala is not yet established (Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2014; Murray et al., 2012; Ochsner & Gross, 2005). Thus far, some studies found decreased connectivity between these regions in relation with neuroticism, whereas others found no effect or an opposite effect (Adelstein et al., 2011; Cremers et al., 2010; Drevets et al., 2008; Etkin & Wager, 2007; Hamilton et al., 2012; Servaas, Riese, et al., 2013a). To our knowledge, no other neuroimaging study has been performed in which specific effects of acute stress on emotion processing have been studied in relation to neuroticism.

Remarkably, on a molecular level it has already been suggested that studies investigating the etiology of neuroticism have focused too much on the simple gene effects and thereby overlooked the importance of gene-environment interactions, leading to contradictory results (Canli, 2008; Ebstein, 2006). One possible explanation for these inconsistent genetic findings is that risk allele carriers only develop higher neuroticism levels when confronted with stressors (Canli, 2008) and hence gene by environment interactions are relevant for this vulnerability path. Indeed, longitudinal research has shown that both positive and negative life stressors are important determinants of the individual's neuroticism levels at a given time, emphasizing the essential role of environmental factors when modeling the pathophysiology of neuroticism (Jeronimus, Ormel, Aleman, Penninx, & Riese, 2013). Notably negative stressful events seem to be responsible for the close link between neuroticism and depression, supporting our finding of enhanced amygdala responsivity under stress in healthy subjects with high scores in neuroticism (Jeronimus et al., 2013). In conclusion, findings from the genetic field confirm the importance of environmental stress in the pathophysiology of neurotic traits in the healthy individual.

Importantly, in our study we found no influence of neuroticism on stress-dependent differences in the neural processing of positive faces. It has been hypothesized that in neurotic individuals a heightened emotional reactivity to positive events would co-occur with a heightened reactivity to negative events, addressing a potential positive side of trait neuroticism (Ormel et al., 2013). Our findings, however, suggest that the heightened emotional reactivity in neuroticism is specific for negative stimuli, in line with the findings of most previous studies (Servaas, van der Velde, et al., 2013b).

Previous experience with the dynamic facial expression task indicated that stress causes a shift of amygdala function to higher levels of sensitivity and lower levels of specificity in healthy subjects (van Marle et al., 2009). In the current study, however, we did not observe this augmentation and generalization of emotion processing under stressful conditions. A possible explanation for this difference is that the prior study

included only female subjects, whereas we included only males. Women are known to have higher neuroticism levels than men (Costa, Terracciano, & McCrae, 2001). Moreover, sex differences are known to influence emotion processing and amygdala function, as well as the acute stress response, possibly causing diverging results in males and females (Cahill, 2006; Kudielka & Kirschbaum, 2005).

Several limitations have to be addressed. Firstly, we only included male subjects. Men and women have even been found to show opposite amygdala responses under noradrenergic arousal, underlining that generalization of our results to females is an important subject for future studies (Schwabe, Höffken, Tegenthoff, & Wolf, 2013). We argue that our study population reflects a representative variety in neuroticism levels for the healthy population since they were not pre-selected on their (extremes in) neuroticism levels. Nevertheless, there is a possibility that we underestimate the effects of neuroticism by studying a relatively resilient group and that we may have found larger effects when comparing two groups with very high and very low neuroticism levels. In addition, we exposed them to only mild stress, reflected by the fact that neuroticism scores did not substantially influence physiological and behavioral stress measures. Although the nature of the stressor is mild, we do believe that the modest, but robust physiological and behavioral stress responses we see in our large sample enable us to make conclusions based on stress-related changes in this population. However, we acknowledge that we cannot make inferences with certainty to neural consequences of more severe stress.

In summary, we show using a well-controlled fMRI study design that the association between neuroticism and amygdala responsivity is dependent on the stressful state of the individual and selective for fearful facial stimuli. This effect constitutes one possible neural mechanism for the increase in stress sensitivity and disease risk associated with high neuroticism levels. In addition, it suggests that coping with negative stressful events should constitute an essential part of treatment for people with high neuroticism levels, when preventing progression to disease. Future studies are recommended to consider this important interaction with environmental stressors when further investigating the neurobiology of neuroticism.

4.5 Supplement

S4.1 Table of fMRI clusters: main effects of condition and task.

Effect	Region	Hemisphere	Cluster size	Peak MNI coordinates			Peak T Value
				x	y	z	
Positive effect of task versus baseline	Inferior occipital gyrus extending into anterior medial temporal lobe	Right	43073	38	-74	-10	44.58***
	Precentral gyrus	Right	4714	48	-2	50	15.71***
	Precentral gyrus	Left	3649	-48	-4	48	13.81***
	Medial frontal gyrus	Right	2055	2	44	-16	10.10***
	Inferior frontal gyrus	Right	253	26	32	-14	7.97***
	Inferior temporal gyrus	Left	252	-64	-4	-18	7.61***
	Medial frontal gyrus	Left	518	-6	-28	60	7.58***
	Cerebellum	Left	56	-28	-64	-50	7.00***
	Caudate	Left	49	-6	20	10	6.92***
	Superior frontal gyrus	Right	182	6	6	62	6.78***
	Superior temporal gyrus	Right	197	44	22	-30	6.43***
	Paracentral lobule	Left	1	-6	-36	64	6.04***
	Caudate	Right	11	10	22	10	5.69***
	Caudate	Left	9	-8	16	10	5.65**
	Precentral gyrus	Right	11	66	-6	26	5.24**
	Precentral gyrus	Right	1	16	-26	62	5.06*
	Inferior temporal gyrus	Right	4	64	-4	-20	4.96*
	Superior medial gyrus	Right	4	8	70	14	4.93*
	Paracentral lobule	Right	1	6	-36	60	4.91*
	Superior medial gyrus	Right	5	8	68	20	4.91*
	Cingulate gyrus	Left	1	-4	0	30	4.82*
	Superior frontal gyrus	Right	4	8	64	26	4.81*
	Superior frontal gyrus	Right	4	14	42	48	4.74*
	Precentral gyrus	Left	2	-64	-8	32	4.74*
	Inferior frontal gyrus	Left	1	-30	30	6	4.66*
	Amygdala	Right	252	22	-4	-16	9.88#
	Amygdala	Left	255	-22	-6	-16	8.80#
	Amygdala	Left	1	-10	-2	-16	4.69#

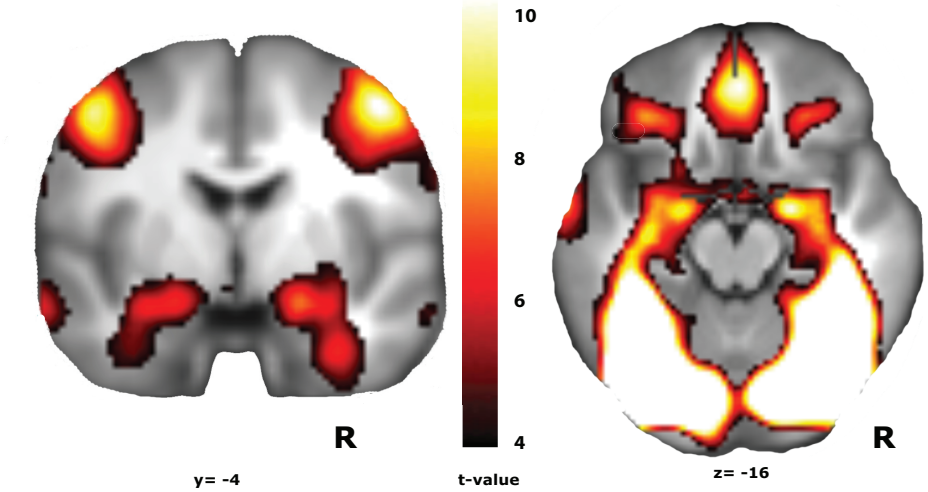
S4.1 Table of fMRI clusters: main effects of condition and task. (continued)

Effect	Region	Hemisphere	Cluster size	Peak MNI coordinates			Peak T Value
				x	y	z	
Negative effect of task versus baseline	Angular gyrus	Right	245	46	-78	30	10.26***
	Hippocampus	Right	49	18	-40	14	8.07***
	Inferior parietal lobule	Left	724	-56	-26	30	7.85***
	Precuneus	Left	289	-8	-54	56	7.82***
	Lingual gyrus	Left	29	-26	-100	-10	7.29***
	Precuneus	Right	185	12	-54	58	7.07***
	Insula	Left	220	-42	-4	8	6.96***
	Hippocampus	Right	31	34	-44	0	6.81***
	Superior occipital gyrus	Left	77	-40	-84	30	6.76***
	Precuneus	Right	1	12	-52	62	6.49***
	Precuneus	Left	1	-6	-60	62	6.43***
	Hippocampus	Left	22	-32	-50	2	6.13***
	Middle frontal gyrus	Left	55	-24	-12	62	5.93***
	Posterior cingulated cortex	NA	58	0	-32	10	5.85***
	Postcentral gyrus	Left	1	-30	-46	64	5.74***
	Superior parietal lobule	Left	1	-18	-58	62	5.68**
	Superior parietal lobule	Left	4	-22	-46	60	5.64**
	Cerebellum	Right	11	22	-92	-28	5.47**
	Cerebellum	Right	6	56	-62	-30	5.32**
	Medulla	Right	8	2	-44	-50	4.91*
	Superior frontal gyrus	Right	56	30	54	18	4.91*
	Supramarginal gyrus	Right	16	64	-52	30	4.89*
	Cerebellum	Left	4	-40	-52	-40	4.85*
	Crus cerebellum	Right	1	58	-46	-46	4.78*
	Thalamus	Left	1	-4	-28	8	4.77*
	Cerebellum	Left	1	-54	-58	-34	4.74*
	Postcentral gyrus	Left	1	-40	-34	46	4.69*
	Middle frontal gyrus	Right	3	30	26	36	4.68*
	Precuneus	Left	1	-6	-72	60	4.68*
Positive effect of condition *(stress > control)*	None	-	-	-	-	-	-
Negative effect of condition *(control > stress)*	None	-	-	-	-	-	-
Positive effect of emotion *(fearful > happy)*	Inferior occipital gyrus	Left	13	-28	-86	-10	4.98*
	Inferior occipital gyrus/ fusiform gyrus	Right	27	30	-86	-6	4.95*
Negative effect of emotion *(happy > fearful)*	None	-	-	-	-	-	-

S4.1 Table of fMRI clusters: main effects of condition and task. (continued)

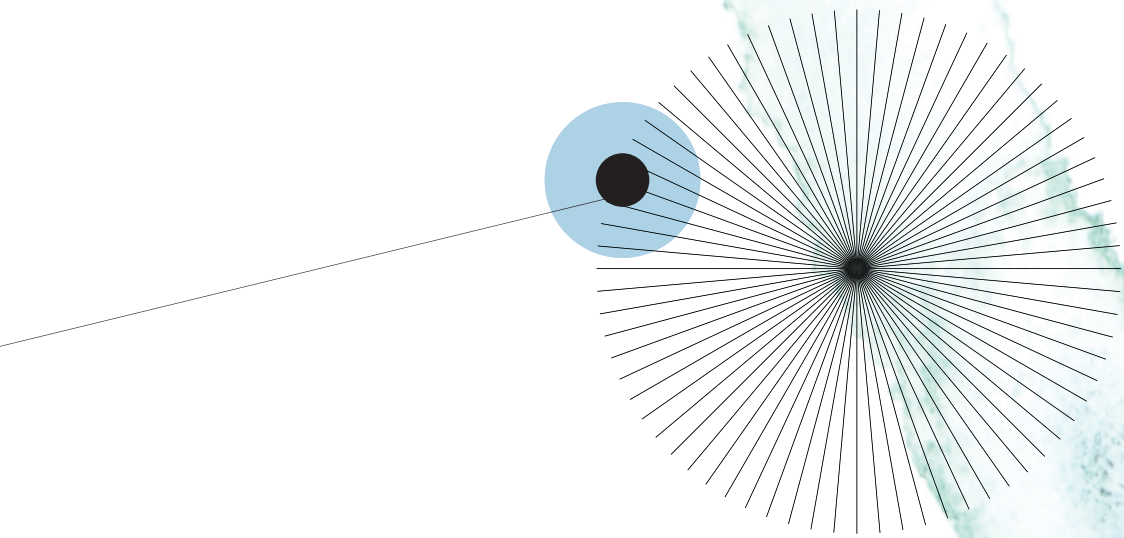
Effect	Region	Hemis-phere	Cluster size	Peak MNI coordinates			Peak T Value
				x	y	z	
Positive interaction stress x emotion *(stress(fear>happy) > neutral(fear>happy)*	None	-	-	-	-	-	-
Negative interaction stress x emotion *(stress(fear>happy) < neutral(fear>happy)*	None	-	-	-	-	-	-

MNI, Montreal Neurological Institute. NA, not applicable. All analyses are FWE-corrected, unless otherwise reported. ***p<0.001, ** p<0.01, * p<0.05, #p<0.001 small volume corrected.



S4.2 fMRI main positive effect of task versus baseline.
T-contrasts are projected on an average normalized T1 over all subjects.

CHAPTER 5
ACUTE STRESS ENHANCES EMOTIONAL FACE PROCESSING IN THE AGING BRAIN



Abstract

Healthy aging has been associated with stable emotional wellbeing and attenuated brain responses to negative stimuli. At the same time, depressive symptoms are common in older adults. The neural mechanisms behind this paradox remain to be clarified. We hypothesized that acute stress could alter emotion processing in the healthy aging brain, and therefore constitute a pathway to vulnerability. Using a randomized, controlled cross-over design we explored the influence of acute stress on brain responses to happy and fearful facial expressions in 25 older adults (aged 60-75) and 25 young (aged 18-30) controls. Groups were matched on trait anxiety and education. Subjects each underwent two separate fMRI sessions involving acute stress or a control procedure. Affective and physiological responses to the stressor were similar between the two age groups. On whole-brain level, we revealed a significant age by stress interaction in the fusiform gyrus, indicating a selective enhancement of neural activity with stress in the elderly only. When specifically aiming our analysis at the amygdala, we found the same stress-related increase in activity in the elderly only. The modulation of amygdala reactivity due to stress correlated with trait conscientiousness in the elderly exclusively. Healthy, older adults showed increased responsivity of brain regions involved in face and emotion processing while stressed, compared with younger adults. These findings suggest that increased reactivity of this neural circuitry after acute stress may constitute one mechanism by which emotional wellbeing during healthy aging could rapidly change into heightened vulnerability for affective disorders.

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5.1 Introduction

Life expectancy is increasing worldwide. By 2050 the number of individuals older than 60 is expected to have doubled (*"World Population Prospects: The 2015 Revision,"* 2015). Unfortunately, these additional years are not always spent in good health. In fact, there is evidence of raising rates of chronic disease and disability in the elderly (Beard, Officer, & Cassels, 2016).

Mental health problems such as dementia and depression are amongst the most significant contributors of disability amongst the elderly (Mathers, Fat, & Boerma, 2008). While most research supports a general age-related decline with respect to cognition, emotional aging is thought to be more complex. In fact, in contrast to the high burden of depression in old age, there is also evidence that healthy aging can give rise to protective psychological effects, reflecting a paradox in emotional aging (Mather, 2012). Healthy older adults, compared with healthy young adults, are better at focusing on positive stimuli, more efficient in regulating their emotions and more biased towards positive memory (reviewed in: (Mather, 2012; Scheibe & Carstensen, 2010)).

In addition, while age-related changes in learning and memory have been extensively studied using state-of-the-art neuroimaging techniques (Grady, 2012; Hedden & Gabrieli, 2004), relatively few studies have investigated the neural correlates of emotional aging. Initial neuroimaging studies have generally confirmed the behavioral findings of an increase in positive emotions in healthy aging and suggest a change in neural processing underlying emotions (Brassen et al., 2012; Clewett, Bachman, & Mather, 2014; Mathieu et al., 2014; Nashiro, Sakaki, & Mather, 2011). For example, attenuated amygdala responses to negatively valenced pictures have been found in older adults when compared to younger adults (Leclerc & Kensinger, 2011; Mather et al., 2004). Correspondingly, increased activity in anterior cingulate and prefrontal regions has been found when processing emotional stimuli, suggesting enhanced cognitive control (Brassen, Gamer, & Büchel, 2011; Chowdhury, Sharot, Wolfe, Düzel, & Dolan, 2014; Leclerc & Kensinger, 2011; Zsoldos, Cousin, Klein-Koerkamp, Pichat, & Hot, 2016).

One critical factor accounting for this paradox of resilience and vulnerability in aging could be the influence of acute stress. Age-related cellular, cerebral and behavioral changes resemble those found in the chronically stressed individual, and acute stress in the aging brain could be "adding fuel to the fire" (Prenderville et al., 2015). Possibly, acute stress could make healthy elderly more at risk to develop symptoms of affective disorders. Thus far, few studies have focused on possible age differences in effects of acute stress or negative mood induction in the laboratory. Some studies find better emotion regulation in older adults after acute stress, whereas other studies find a

decline of positive emotions or no age differences at all (Dijkstra, Charness, Yordon, & Fox, 2009; Kliegel, Jäger, & Phillips, 2007; Larcom & Isaacowitz, 2009; Wrzus, Müller, Wagner, Lindenberger, & Riediger, 2014). Effects on physiological stress parameters are also unclear (Uchino, Birmingham, & Berg, 2010). Unfortunately, in these studies the role of potential confounders such as co morbidity and use of medication are mostly not taken into account. Moreover, we and others have shown that neural stress reactivity may be influenced by personality (Dahm, Schmierer, Veer, & Streit, 2017; Everaerd, Klumpers, van Wingen, Tendolkar, & Fernández, 2015; Servaas, Riese, et al., 2013a). Personality is generally investigated using a set of personality traits based on for example the "five-factor model", including neuroticism, extraversion, openness to experience, agreeableness and conscientiousness (McCrae & Costa, 1999). Importantly, these traits change across the life span: while openness and extraversion decline with age, conscientiousness increases during adulthood and neuroticism stays relatively stable (Lucas & Donnellan, 2011). Thus, personality should be considered, since age-related changes in personality traits may influence potential age-related differences in the stress response. Lastly, to our knowledge, no prior study has investigated age differences in neural activity during experimental stress induction procedures.

In the current study, we therefore aimed to unravel the influence of acute stress on neural emotion processing in healthy aging, using a well-established experimental stress induction procedure. We hypothesized that, in line with previous research demonstrating emotional resilience with increasing age, healthy older adults would show attenuated responsivity of brain regions involved in emotion processing, in particular the amygdala, compared to healthy young adults under standard conditions. However, we anticipated that this age-related difference would become smaller or even disappear after the administration of acute, psychological stress, reflecting the paradox with enhanced vulnerability in healthy aging. Since we previously found that individual differences can also influence the neural stress response within this paradigm (Everaerd et al., 2015), we additionally explored the impact of personality traits.

5.2 Methods and materials

Participants

We included 25 young (aged 18-35) and 25 older (aged 60-75) healthy men (described in table 5.1). Young adults were individually selected from an existing (n=120) database (Everaerd et al., 2015). We carefully matched these younger subjects to the older adults based on similar trait anxiety scores and educational levels, since we estimated these

Table 5.1 Demographic characteristics of the study population.

	Younger adults N = 25	Older adults N = 25	p-value
Age (years)			
Range	18-30	60-75	
Mean (SD)	21.5 (2.5)	66.7 (4.3)	<.001
Educational attainment			NS
Primary school (%)	0	1 (4)	
Lower secondary (%)	3 (12)	2 (8)	
Intermediate secondary/ College degree (%)	9 (36)	6 (24)	
Higher secondary/ University degree (%)	13 (52)	16 (64)	
Trait anxiety score* (SD)	32.8 (6.3)	32.6 (8.8)	NS
NEO-FFI scores* (SD, range)			
Altruism	42.0 (3.4, 37-48)	44.2 (3.9, 36-52)	0.037
Conscientiousness	41.1 (5.5, 29-50)	44.7 (4.7, 35-53)	0.018
Extraversion	44.8 (6.5, 30-53)	39.0 (5.2, 30-50)	0.001
Neuroticism	26.4 (7.0, 14-40)	25.3 (6.7, 14-39)	NS
Openness	39.4 (6.8, 28-52)	37.2 (6.1, 28-51)	NS
Mean baseline cortisol (nmol/l) (SD)	12.3 (6.5)	13.2 (5.3)	NS
Total brain volume (ml) (SD)	1373.9 (84.1)	1266.6 (88.0)	<.001
Amygdala volume (ml) (SD)	2.7 (0.5)	3.0 (0.4)	.064
Amygdala volume as percentage of total brain volume (%) (SD)	0.20 (0.03)	0.23 (0.04)	<.001

* All scores are in the normal range for a healthy male population (Creamer et al., 1995; Hoekstra et al., 1996). All p-values below p<0.1 are reported.

factors to be potential confounders of age-related differences in neural activity (Demenescu et al., 2014; Rönnlund, Nyberg, Bäckman, & Nilsson, 2005).

Procedure

All participants took part in a two-session study with a randomized, counterbalanced order of the session type (stress or control; fig. 1.3). We have described this procedure in detail elsewhere (Everaerd et al., 2015; Henckens et al., 2016) and it was extensively standardized in order to create a highly similar experimental setting for all participants. Details of the data acquisition and processing procedures can be found in the supplemental information (S5.1). Sessions were separated by on average 13 days (minimum of 5 days). All testing took place between noon and 6 pm with the aim of profiting

from more stable hormone levels to limit the influence of the diurnal rhythm on our hormone assessments. In short, during one hour of pre-scanning preparation, participants received information about the study, practiced the tasks they would later have to perform in the scanner, and watched a relaxing nature documentary (Attenborough, 2010). Next, during the stress session a state of acute stress was induced by showing highly aversive movie clips in the MRI scanner (Cousijn et al., 2010; Hermans et al., 2011; van Marle et al., 2009). These clips consisted of scenes of a movie (Noé, 2008) containing extremely aggressive behavior and violence against men and women. During a separate control session, neutral, non-arousing scenes of another movie (Fontaine, 2005) were shown. The stressful and the neutral movie clips both had a duration of 10 minutes and were similar in the amount of speech, human (face) presence, luminance, environment, and language. The participants were asked to watch the movie clips from an eye-witness perspective.

Immediately after the first movie clips, subjects performed the dynamic facial expression task, which consisted of passive viewing of photographs of emotionally neutral faces, morphing into two different emotion types: fearful or happy facial expressions (Ekman & Friesen, 1976). The morphing faces were presented in a block design (three blocks of each emotion, 25 s per block, 0.5 s per face, avoiding adjacent blocks of the same emotion), interleaved with blocks of fixation cross for baseline reference purposes (three blocks, 25 s per block). Reaction times were measured to evaluate general attention, expressed as mean time to respond to the fixation cross. After this task, the subjects participated in other studies with different questions at issue of which the results will be reported elsewhere. A structural scan was obtained at the end of the stress session. The duration of this multi study scanning was approximately 105 minutes per session. In between sessions, participants completed several self-report questionnaires, containing the Dutch versions of the trait/state anxiety inventory (Spielberger et al., 1970) and the NEO-FFI (McCrae & Costa, 1999).

Imaging Data Analysis

We used a factorial ANOVA as implemented in SPM8 with stress condition and emotion type as within-subject factors, and age group as between-subjects factor. This model resulted in statistical parametric maps, which were superimposed upon the mean anatomical image across all subjects for localization purposes. Our statistical threshold for these voxel-wise analyses was set at $p<0.05$ Family-Wise Error (FWE) corrected for multiple comparisons with Gaussian random field theory as implemented in SPM8.

Since we were interested a priori in differences in amygdala responses between the two groups, we also performed small volume corrected analyses (threshold $p<0.05$

FWE corrected) using a standard anatomical atlas for the bilateral amygdala (Tzourio-Mazoyer et al., 2002). Based on previous literature reporting confounding influences of local brain atrophy on functional MRI analyses in healthy aging, we additionally made use of individual masks of the amygdala to extract beta-values from the individual parameter estimate maps (Kalpouzos, Persson, & Nyberg, 2012). To this end we created individual masks by means of an automatic segmentation using the FIRST module of FSL (First version 1.2 (www.fmrib.ox.ac.uk/fsl/first/index.html) (Patenaude et al., 2011) in FSL version 4.1.9 (www.fmrib.ox.ac.uk/fsl), developed by the Analysis Group, FMRIB, UK). This method is based on Bayesian statistical models of shape and appearance for bilateral amygdala from 317 manually labelled T1-weighted magnetic resonance images. Visual inspection of the segmented amygdala masks projected onto the T1-weighted MRI scans was performed using the software MRIcroN Version Beta 7 (www.mricro.com/mricron). After automatic segmentation, volumes were calculated using the MarsBar SPM toolbox version 0.42 (http://marsbar.sourceforge.net).

Other Data Analysis

All other data (baseline variables, questionnaire scores, heart rate, heart rate variability, blood pressure, cortisol levels, and PANAS scores) were analyzed in SPSS 19 (IBM). Stress * time (before and after stress induction) repeated measures ANOVAs with group as a between subjects factor were used to evaluate stress responses in behavioral and physiological measures. The heart rate was calculated as 60/mean interbeat interval and heart rate variability as the root mean squares of successive differences between successive interbeat intervals. Offline artifact correction and analysis of the heart rate frequency and variability were done with in-house software.

For correlation analyses parametric tests were used as default (Pearson correlations). Only for correlations with reaction times, response accuracy, heart rate and heart rate variability nonparametric tests were used (Spearman correlations) since these variables were not normally distributed. All reported analyses were performed with all subjects for whom data were available included. Removing possible outliers (z-scores>2.5) significantly changed our results for heart rate (variability) and extracted amygdala beta values only. Results for these analyses were reported with outliers excluded. Significance level was set at $p<0.05$. A linear regression analysis with one model containing all NEO-FFI subscales as predictor of stress-related changes in amygdala responses was performed to explore whether there were any personality-related moderators significantly predicted amygdala responses while taking the other subscales into account. Subsequently, separate correlation analyses for each predictor were performed to verify associations. Bonferroni corrections were applied for these correlational analysis (Curtin & Schulz, 1998).

5.3 Results

Study population

One subject was excluded during the screening procedure because of previously undetected hypertension and cardiac arrhythmia. A second subject decided not to continue his participation during the first scanning session. Both subjects were older adults and were replaced in order to maintain a sample size of 25 subjects per group.

Although we matched our two age groups on trait anxiety levels, their personality scores were still different on some subscales, consistent with current literature suggesting common personality changes with increasing age (table 5.1) (Roberts, Walton, & Viechtbauer, 2006). In addition, amygdala volumes were relatively larger in older than young subjects.

Reaction times to the fixation cross presented in between the blocks did not significantly differ between the two groups (age group*stress: $F(1,45)=0.7$, $p=0.409$), main effect of age: $F(1,1)=0.2$, $p=0.688$; mean reaction times across sessions in young adults = 667.4 ms, older adults = 702.0 ms), suggesting similar levels of attention between the two age groups.

Stress induction

A similar state of mild, acute stress was induced in both age groups, confirmed by significant changes in heart rate and negative affect ratings (see S5.2 for details).

Cortisol levels (as a proportion of mean cortisol levels measured at rest at home) only showed a main effect of time ($F(1,48)=23.9$, $p<0.001$; baseline = 1.0, after task = 0.8). There were no interactions, suggesting that diurnal fluctuations in cortisol levels were stronger than the influence of our mild stressor.

Mean blood pressure levels, heart rate variability and subjective positive affect ratings differed significantly between the two age groups, but our stressor did not significantly influence these pre-existing differences during the task.

fMRI: Main effects

The viewing of faces, independent of emotion type or session, activated the expected network of brain regions across both groups, extending from the superior occipital gyrus to the fusiform gyrus, precentral gyrus and medial temporal lobe, including the amygdala. Deactivations compared to baseline were observed in the supramarginal gyrus and middle occipital gyrus (all $p_{FWE}<0.05$, S5.3).

Group differences in task activations were found in the occipital cortex and fusiform gyrus, where the younger subjects showed higher activation levels than the older adults (all $p_{FWE}<0.05$, S5.3). There were no regions that elicited more activation in older than younger adults.

Across both age groups there were no brain regions that showed more activation in the stress session than in the control session. In addition, there were no brain regions with more responsivity in the control than the stress session.

fMRI: Interaction effects of age and stress

A three-way interaction between stress, emotional valence and age did not reach significance. However, we observed a significant age by stress interaction in the lingual gyrus extending into the fusiform gyrus (Table 5.2, Figure 5.1). In this region older adults showed a stress-related increase in activity compared to the younger adults.

We explored this interaction by investigating stress effects per age group. Here we found a significant increase of activity in the stress condition compared to control in the older adults in a more anterior region reaching into the parahippocampal gyrus (Peak MNI coordinates -32 -9 -30, p=0.036 cluster level corrected). When applying a small volume correction for the interaction effect in the elderly, we indeed found a stress-related increase of activity in the lingual/ fusiform gyrus (Table 5.2). In the younger adults, we only found a significant effect in the opposite contrast (stress<control) in the postcentral gyrus (Peak MNI coordinates 63 -2 32, p=0.030 cluster level corrected). When applying a small volume correction for the interaction effect in this young group, we did not find any change of activity in this region (Table 5.2).

Using voxel-wise analysis we did not find an effect in the amygdala, our initial region of interest. Therefore, we then used the targeted analysis of extracting beta values from the individually defined, anatomical amygdala of each single subject. This technique enabled us to directly compare the change of activity in the two sessions in the young and older adults separately, with an even greater certainty that normalization differences do not affect our result. Moreover, bilateral amygdala volume did not correlate with amygdala BOLD responses both within and across the two groups, indicating that the group difference in amygdala volume in our study population would not drive any group effects in amygdala responsivity. Although we had a clear a priori hypothesis of stress-related increase of amygdala responsivity in the elderly, for completeness we still tested potential interaction effects. There were no significant interactions between age group, session or valence (age group*stress*valence: $F(1,45)=0.2$, $p=0.643$; age group*stress: $F(1,45)=1.2$, $p=0.274$; age group*valence: $F(1,45)=0.5$, $p=0.502$;

Table 5.2 Interactions of age and stress. MNI, Montreal Neurological Institute.

Effect	BA	Region	Hemi-sphere	Cluster size	Peak MNI coordinates			Peak F/T Value
					x	y	z	
Positive interaction age x stress ***(old (stress>control) > young (stress>control))***	19	Lingual gyrus/ fusiform gyrus	Right	825	32 24 22	-65 -60 -39	0 -4 0	4.53**
Negative interaction age x stress ***(old (stress>control) < young (stress>control))***		None	-	-	-	-	-	-
Stress effect in young* ***(stress<control)***		None	-	-	-	-	-	-
Stress effect in old* ***(stress>control)***	19, 36, 37	Fusiform gyrus	Left	104 97 10 16	24 30 34 27 33	-44 -65 -63 -54 -39	0 2 -1 -9 -6	-

BA, Brodmann's area. **p<0.01 cluster level corrected with an initial whole brain voxel-wise threshold of *p* < .001 uncorrected for multiple comparisons. Small volume corrections for the bilateral anatomical amygdala (AAL atlas (Tzourio-Mazoyer et al., 2002)) did not yield any additional clusters. *Effects are masked for the interaction effect; therefore, no statistics are performed.

stress*valence: F(1,45)=0.3, p=0.595). However, amygdala responsivity across both sessions was lower in older than younger adults (F(1,45) = 4.4, p = 0.041; young adults = 0.2, old adults = 0.1). Similar effects were found when adding amygdala volume as covariate, again indicating these effects were not driven by volumetric changes. Given our a priori hypothesis of stress as enhancing factor for amygdala reactivity in older adults, we further explored possible group differences in amygdala responsivity to the different stimuli. Interestingly, here we found a significant increase of amygdala responsivity under stress within the group of older adults, but not in the young adults (Figure 5.2). We additionally explored valence-specific effects and found that this difference was mainly driven by a stress-induced increase in the response to fearful faces (S5.4). Because of the absence of a clear hypothesis for this valence-specific finding and the non-significant interactions, this should be interpreted with caution.

Individual differences in stress responses

Finally, we were interested in age-specific differences in personality traits that could be associated with changes in amygdala responsivity under stress. Interestingly, we

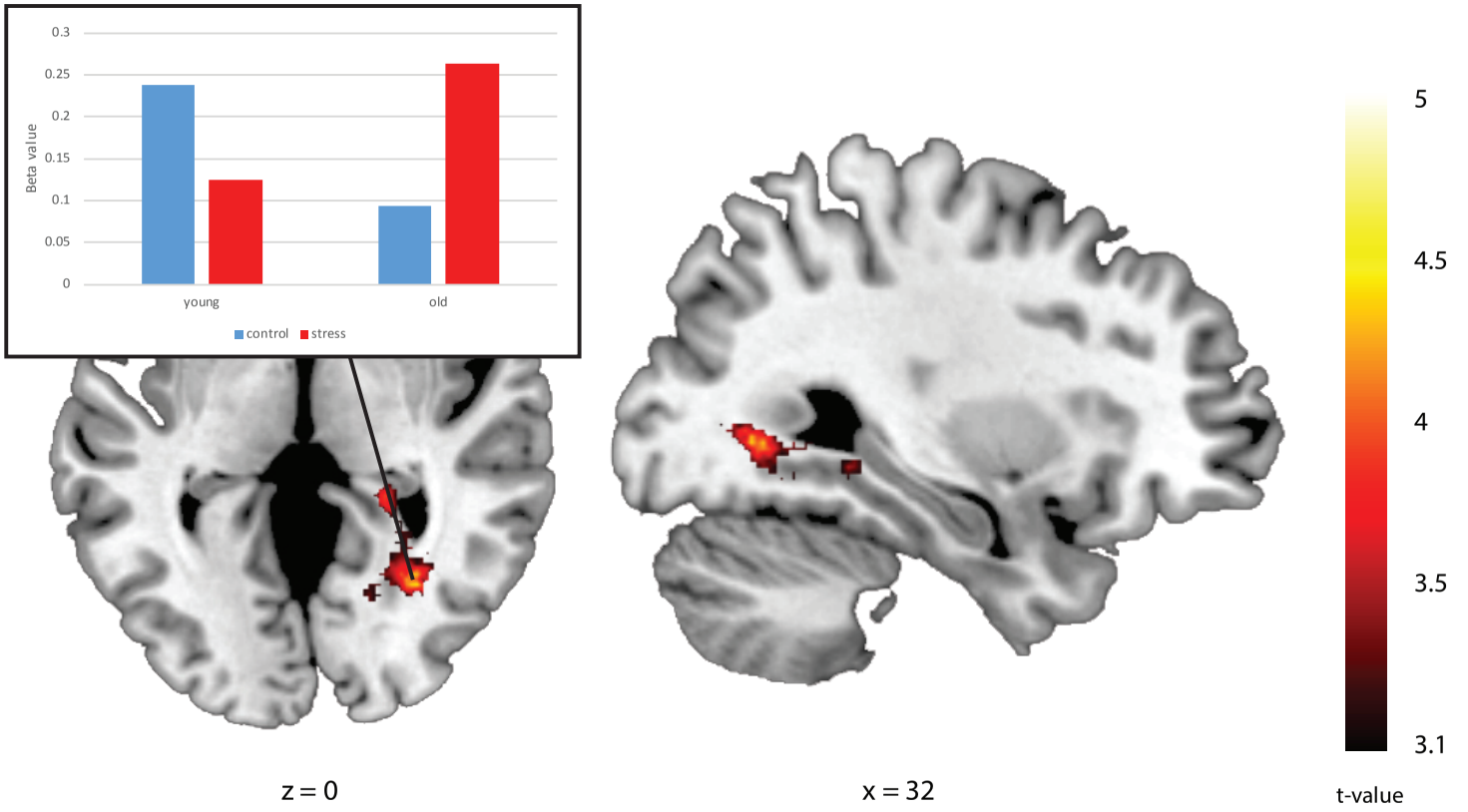


Figure 5.1 Positive interaction between age and stress in the lingual gyrus extending into fusiform gyrus (indicated by arrows), representing stress–related enhancement of activity in the elderly only.
The image is thresholded at $p_{uncorrected} < 0.001$ and masked for the cluster level significant effect.
The bar graph depicts mean beta values within the significant cluster for the age groups and sessions.

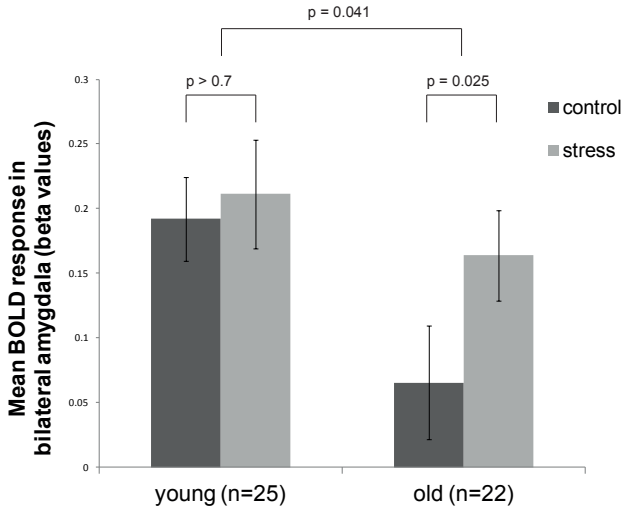


Figure 5.2 Mean amygdala responsivity in the two sessions per age group.
Amygdala beta values were extracted from the anatomical bilateral amygdala, using individual masks for every subject.

found that of the five subscales in the model, only conscientiousness levels predicted a stress-related difference in amygdala responsivity within the elderly (b= -0.541, t(16) = -2.207, p=0.042). There were no significant predictors of amygdala responsivity in young adults. We verified this result by correlating the five different NEO-FFI subscale scores to the difference in amygdala responsivity between the stress session and control session. Here, a negative correlation between conscientiousness and the impact of stress on amygdala responsivity in older adults confirmed our finding (r(20) = -0.534, p=0.007), but not the young adults (r(23) = -0.042, p=0.840) (Figure 5.3, S5.2 and S5.5). After applying Bonferroni correction for the five subscales (significance level p=0.05/5 = 0.01) this result remains significant. The difference between these correlations showed a trend towards significance, indicating a more negative association in elderly (z=1.81, p=0.07).

5.4 Discussion

To the best of our knowledge, this is the first study that investigates whether older age is associated with changed neural reactivity to acute stress. We found that overall physiological stress reactions were similar across both age-groups, suggesting that our type of stressor had a similar impact across subjects of different ages. Secondly, we replicated earlier findings that positive affect was generally higher in healthy older than younger adults. Interestingly, higher positive affect in our elderly was accompa-

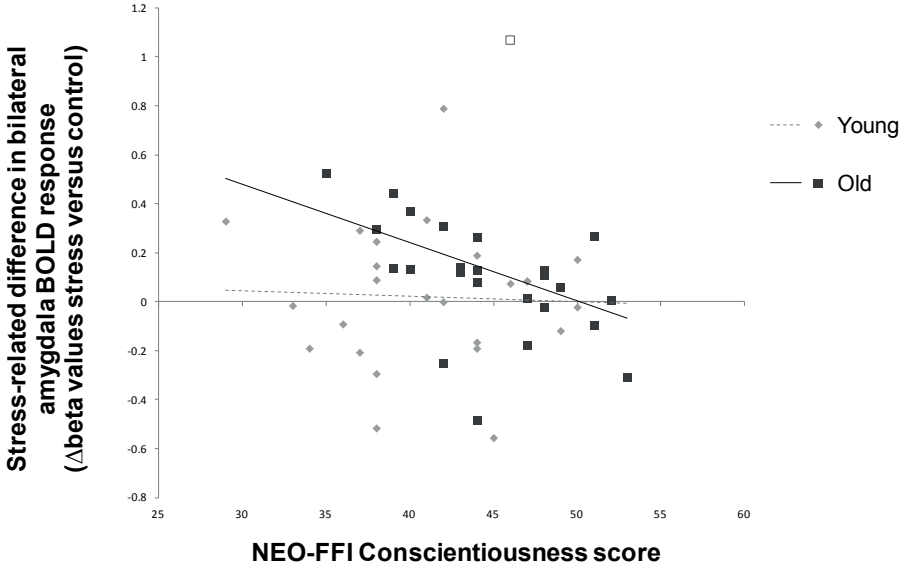


Figure 5.3 Conscientiousness was negatively correlated (p=0.007) with the stress–related change in amygdala responsivity in older adults only.
The open square represents an outlier (z-value 3.4) that was excluded from the statistical analysis.

nied by an attenuated amygdala response to emotional facial stimuli in older adults. Fundamentally, we observed that acute stress selectively enhanced neural activity in visual processing regions as well as the amygdala the elderly, bringing their neural responses to the same level as young adults. Although our study design does not permit statements about causality, this shift in neural activity in the amygdala appears to be influenced by the personality trait of conscientiousness in the older adults only.

These findings support previous research on age-related changes in brain activity underlying the positivity bias in healthy aging, in the sense that under standard conditions the healthy aging brain seems less susceptible to emotionally salient information, than the healthy younger brain (Brassen et al., 2011; 2012; S. Dolcos, Katsumi, & Dixon, 2014; Mathieu et al., 2014). However, notably under acute psychological stress, we found that even the healthy aging brain becomes significantly more reactive to emotional facial input. This is a novel finding, confirming the significant impact of stress in the already challenged aging brain (Prenderville et al., 2015). Interestingly, aging is normally accompanied by an age-related reduction in occipital activity coupled with increased frontal activity (the posterior-anterior shift in aging, PASA) (Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008). The PASA phenomenon is thought to reflect sensory decline in aging accompanied by prefrontal compensation, and is also found for emotional

stimuli (St Jacques, Dolcos, & Cabeza, 2010). Although we did not find an increase of frontal activation, our results suggest that the PASA phenomenon could be influenced by acute stress. More research is needed to better understand PASA changes under stress, and in particular role the amygdala has in this phenomenon.

With our paradigm we induced only mild physiological stress and we did not find significant stress effects in all physiological parameters. Since our stress induction paradigm has been previously used in a larger sample size where it lead to more significant stress effects, we believe that this is most likely due to our relatively small sample size in combination with the moderate nature of the stressor
(Everaerd et al., 2015; Henckens et al., 2016). Interestingly, bodily stress responses in our study were very similar between our younger and older adults. The few studies investigating the consequences of acute psychological stress in older adults show contradicting results for cortisol responses, heart rate and blood pressure reactivity (Kudielka et al., 2009; Uchino et al., 2010). Of note, gender differences could potentially influence these findings. For example, the Trier Social Stress Test was found to elicit blunted heart rate responses in older versus younger adults, but the effect was largely driven by women (Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). In addition, not all studies investigating autonomic reactivity after experimental stress take the potential influence of co morbidity and medication use into account (Uchino et al., 2010). Lastly, different types of experimental stressors could have a dissimilar impact on young and old adults (Uchino et al., 2010). By making use of emotional movie clips, we aimed to limit these potential age-related influences (Tsai, Levenson, & Carstensen, 2000), but the present results should be replicated using other ecologically valid stress situations. For example, contextual features of testing environments have been found to differentially influence cortisol responses in young versus older adults (Sindi et al., 2014). Also, age differences in violent movie or video game exposure could influence differences in stress responses after violent movie clips. Further research is needed, particularly with respect to gender differences and influence of different types of stressors, to gain better understanding of the role of physiological stress responses in age-related differences in emotion processing.

Importantly, previous studies have suggested that the positivity bias is due to a selective decrease in the neural processing of negative information with age (Mather, 2012). In our study however, we did not find an interaction with stimulus type, meaning that for young as well as for older subjects there were no differences in brain activity in response to happy versus fearful facial stimuli. Since a similar task in young, healthy females did elicit valence-specific responsivity of the amygdala, sex differences could be one reason why we did not find this effect in our male population (van Marle et

al., 2009). In addition, older adults have been found to have impaired recognition of basic emotions in facial expressions compared to younger adults (Ruffman, Henry, Livingstone, & Phillips, 2008). Our dynamic facial expression task could have been too challenging for older adults to successfully distinguish different types of salient facial expressions.

Furthermore, we did not observe a direct effect of acute stress on amygdala responsivity in young adults. This finding is in line with previous findings from our larger sample of 120 young adults, where we found no stress effect on amygdala responsivity in the study population as a whole, but concluded that individual differences moderated amygdala responsivity to emotional faces under stress (Everaerd et al., 2015; Henckens et al., 2016). Here, we found that in older adults only trait conscientiousness modulated the amygdala response to stress. This finding is of particular relevance for the elderly, since high levels of conscientiousness have repeatedly been associated with attenuated cognitive decline in aging (Terracciano et al., 2013; Wilson et al., 2015; Wilson, Schneider, Arnold, Bienias, & Bennett, 2007). Importantly, trait conscientiousness also seems to interact with environmental stress when conveying a risk for affective symptoms. Low levels of conscientiousness were related to higher anxiety levels in combination with stressful life events in a sample of older adults with late-life depression (van der Veen et al., 2016). Our finding may thus constitute a neural mechanism for the resilience associated with conscientiousness in older age.

The main strength of our study is its novelty in using a well-investigated fMRI stress induction procedure to demonstrate stress-related changes in neural emotion processing in older adults, which to our knowledge is the first to do so. An important limitation of our study is the inclusion of only men. The aging brain shows sex-specific changes in stress responsivity, indicating that generalization to women should be done with caution (Bale & Epperson, 2015). Secondly, the cross-sectional nature of our study cannot exclude possible cohort effects. For example, age-related changes in frontal activity has shown opposite directions when using different study designs: while cross-sectional analyses were suggestive of age-related frontal over recruitment, the longitudinal analyses revealed frontal under recruitment with advancing age (Nyberg et al., 2010). Since group differences in educational attainment are an important confounding factor in cross-sectional studies investigating aging (Rönnlund et al., 2005) and differences anxiety levels could bias our results as well, we aimed to limit these influences by matching our groups on educational level and trait anxiety. Finally, a larger sample with a more continuous age range would have provided additional information on the role of neural development and is recommended for future studies investigating neural stress sensitivity in aging.

Increased understanding of acute stress responses in healthy aging could help identify elderly at risk for mood and anxiety disorders when confronted with life stressors, as recently has been demonstrated in young adults (J. R. Swartz, Knodt, Radtke, & Hariri, 2015). Moreover, individual differences in amygdala (re)activity may predict treatment responses in depression, highlighting the importance of gaining insight in the age-related differences in the dynamics of emotional aging (M. L. Phillips et al., 2015). Interestingly, in older adults, neural responses to angry faces have already been found to be associated with suicidal behavior (Vanyukov et al., 2015). This is highly relevant, since suicide rates are traditionally highest in older males compared to other groups of society (World Health Organization, 2014).

In conclusion, in this study we found evidence for attenuated emotional facial processing in the healthy aging brain, which seemed reversed during acute stress induction and dependent on conscientiousness levels in the older adults. Understanding how our work can be translated to more vulnerable individuals should be an important goal of future research.

5.6 Supplement

S5.1 Supplemental methods and materials

Participants

Candidates for participation were recruited using a local participant database and advertisements at the Radboud University (including a department for higher education for older adults). Initial screening was conducted by self-report questionnaires before participation. Participants were excluded if they reported a history of somatic disease potentially affecting the brain, current or past psychiatric or neurological disorder, relevant medication (e.g. beta-blockers, benzodiazepines) or illicit drug use during the preceding 6 months, history of substance abuse, current or past alcohol dependence, or MRI contraindications. Young women, and for matching purposes therefore older women, were excluded because the menstrual cycle is known to influence correlates of the stress response (Kirschbaum et al., 1999; Ossewaarde et al., 2011).

The older adults additionally underwent a brief screening procedure on the first day of participation. A physician obtained a concise medical history, cognitive screening, physical examination and electrocardiogram in order to exclude subjects with possible undetected cognitive problems, and cardiovascular or pulmonary disease that could be at increased risk during stress induction. Participants received up to 60 Euros reimbursement for full participation. Written informed consent was obtained after complete description of the study to the subjects. The study was approved by the local ethics committee (CMO Region Arnhem-Nijmegen, the Netherlands).

MRI Acquisition

MR data of were acquired on a 1.5 T Avanto MR scanner (Siemens, Erlangen, Germany) at the Donders Institute in Nijmegen, the Netherlands. A series of 129 T2*-weighted functional images were acquired using gradient echo-planar imaging (EPI) with the following parameters: 32 oblique transverse slices, voxel size = 3.5 X 3.3 X 3.3 mm, repetition time (TR) = 2.34 s, flip angle $\alpha = 90°$, echo time (TE) = 35 ms. A 3D magnetization-prepared rapid gradient echo (MPRAGE) anatomical T1-weigthed image was acquired for normalization purposes (176 slices, 1.0 mm isotropic, TR = 2730 ms, TE = 2.95 ms).

Sampling of stress parameters

During each session, two saliva samples were obtained for analysis of free cortisol: before stress induction (t=-15 min.) and immediately after the task (t=18 min.). In order to get a baseline measure of cortisol without any effects of our experimental procedures (including the scanning), participants were asked to collect two extra samples at the same time-of-day on the day before the visits. The average of both samples taken at home was used as baseline for the statistical analyses.

Before scanning (t=-15 min.), resting blood pressure measures of participants were obtained using a standard automatic blood pressure device. Blood pressure was also measured immediately after the task (t=18 min.), using a semi automatic MR-compatible blood pressure device. Changes in affect during the scanning procedure were assessed at these same time points using the Positive and Negative Affect Scales (Watson et al., 1988). Heart rate was continuously assessed during scanning by the use of an MR-compatible pulse oximeter.

Image Data Processing

MR data quality checks were performed by visual inspection of the structural and functional scans, spike checks, signal-to-noise ratio plots and assessment of movement. One older adult exceeded our movement threshold of 3.5mm (one voxel) by 1.2 mm. Since excluding this subject did not change any of our between-group effects, we decided to keep this subject in the final analysis. Functional MRI data were analyzed using SPM8 (UCL, London). For preprocessing, voxel time series were interpolated to correct for non-simultaneous slice acquisition within each volume and were corrected for three-dimensional motion. After realign-

ment and spatial co-registration to the structural image, all functional images were normalized. We used DARTEL, a high-dimensional diffeomorphic registration, to create a mean template of our subjects (older and younger adults together) and normalize our data to this common template before normalization into standard stereotactic space (Montreal Neurological Institute [MNI]152 T1-template) (Ashburner, 2007). By means of this procedure we aimed to avoid group differences in normalization, that could occur since existing anatomical variability between our groups (e.g. due to gray matter atrophy and sulcal expansion in the older subjects) could systematically bias the spatial normalization procedure and thereby potentially cause group differences in activation (Samanez-Larkin & D'Esposito, 2008). Smoothing was performed with a Gaussian kernel of 8 mm full-width at half-maximum. Subsequently, a General Linear Model was used to characterize voxel-wise signal covariation with task parameters for voxels encompassing the brain mask.

The two emotion types (fearful and happy) were modeled separately as boxcar regressors and convolved with the canonical hemodynamic response function implemented in SPM8. Six realignment parameters were included to model potential movement artifacts. Contrast parameter images were then generated at the single subject level (emotion type compared to fixation). These individual parameter estimate maps were statistically scrutinized at a second level.

S5.2 Supplemental results

Stress parameters

A repeated measures ANOVA across both groups showed a session * time interaction effect for subjective negative affect ($F(1,47)=8.0$, $p=0.007$; $\Delta_{\text{after task - baseline}}$ in stress = 2.5, control = -0.1). In line with the emotional wellbeing hypothesis in elderly, subjective positive affect was generally higher in older adults ($F(1,47)=4.6$, $p=0.037$; young adults = 28.6, old adults = 31.9), independent of session. There was also a main effect of time ($F(1,47)=31.0$, $p<0.001$; $\Delta_{\text{after task - baseline}} = -2.6$), indicating a general decline in positive affect during both sessions in both groups. Negative affect changes did not differ between the two groups (interaction group * session * time: $F(1,47)=0.8$, $p=0.377$).

Heart rate during the movie showed a main effect of session ($F(1,46)=21.3$, $p<0.001$; stress = 66.8 BPM, control = 62.4 BPM). During the task the difference in heart rate between the two sessions was at trend significance level ($F(1,46)=3.3$, $p=0.076$; stress = 64.6 BPM, control = 63.0 BPM). There was no difference between the two groups (interaction group * session: $F(1,46)=0.9$, $p=0.339$; group effect: $F(1,46)=0.005$, $p=0.941$).
Heart rate variability was significantly lower in older adults than younger adults during both the movie as the task, independent of stress ($F(1,42)=9.2$, $p=0.004$; mean of movie and task in young adults = 68.8 ms, in old adults = 48.9 ms). During the movie, there was also an interaction between session and group ($F(1,42)=5.3$, $p=0.026$). Older adults showed an increase of heart rate variability (mean 27.4 ms) under stress, whereas young adults showed a decrease (mean -12.1 ms). There was no such interaction during the task ($F(1,42)=0.2$, $p=0.627$).

Although this effect was not significant (interaction session * time: $F(1,48)=0.97$, $p=0.329$), cortisol levels decreased more in the control session than in the stress session in both groups (young adults: in control session -0.26, in stress session -0.23; old adults: in control session -0.22, in stress session -0.13). Importantly, this change did not significantly differ between the two groups (interaction group * session: $F(1,48)=2.2$, $p=0.147$; group * time: $F(1,48)=0.6$, $p=0.461$; group * session * time: $F(1,48)=0.3$, $p=0.598$). However, there was a trend effect for slightly higher cortisol levels in the elderly ($F(1,48)=4.0$, $p=0.052$; young adults = 0.8, older adults = 1.1). Critically, there were no interactions between group and stress, and there is therefore no indication that potential group differences in cortisol levels influenced the age differences in neural stress responsivity we found.

Systolic and diastolic blood pressure levels were overall higher in the older adults than younger adults (systolic: $F(1,48)=28.8$, $p<0.001$; younger adults = 116.6 mmHg, older adults = 130.3 mmHg; diastolic:

F(1,48)=56.4, p<0.001; younger adults = 67.6 mmHg, older adults = 80.1 mmHg), but did not show any significant interactions with session (interaction group * session * time for systolic blood pressure F(1,48)=0.8, p=0.365; diastolic blood pressure: F(1,48)=3.1, p=0.087). Blood pressure levels varied as a function of group and time (corresponding to change in measurement position from sitting to supine), but not session. There was a significant interaction of time * group for systolic blood pressure (F(1,48)=33.0, p<0.001; Δafter task - baseline in young adults = -18.3, older adults = -2.9), indicating smaller positional changes (sitting before scanning versus lying during scanning) in systolic blood pressure in older adults than younger adults.

Individual differences in stress responses

Importantly, our effect appeared to be relatively specific to the amygdala. No significant clusters were found when correlating conscientiousness to brain responsivity in an additional voxel-wise, whole brain analysis. Furthermore, when testing the correlation of conscientiousness with the enhancement of activity under stress in the fusiform gyrus in older adults, we did not find this correlation ($r(23) = -0.275$, $p = 0.184$). Additionally, we ruled out that this correlation was due to session order effects (for example if subjects low in conscientiousness more often had a stress session as their first session). Mean conscientiousness scores were similar between the subjects who started with stress and those who started with the control condition (younger adults: t(23)=0.1, p=0.912; older adults: t(23)=-0.5, p=0.612).

We also checked whether conscientiousness correlated with any of the behavioral and physiological stress parameters. Only the change in cortisol levels due to stress induction correlated with conscientiousness in the older adults ($r(22) = 0.534$, $p = 0.007$). This was a positive correlation, suggesting that higher conscientiousness was associated with a larger stress-related increase in cortisol levels (S5.6). There was no significant correlation between stress-related differences in amygdala responsivity and cortisol levels ($r(22) = -0.153$, $p = 0.496$).

Finally, we verified that the age by stress interaction we found in the whole brain analysis in the lingual gyrus extending into the fusiform gyrus was not the consequence of potential group differences in conscientiousness levels. To this end, we added the covariate of individual conscientiousness scores to the existing model. Correcting for conscientiousness yielded the same MNI coordinates with a cluster size of 833 (cluster level significant), confirming that conscientiousness levels did not drive the interaction between age and stress.

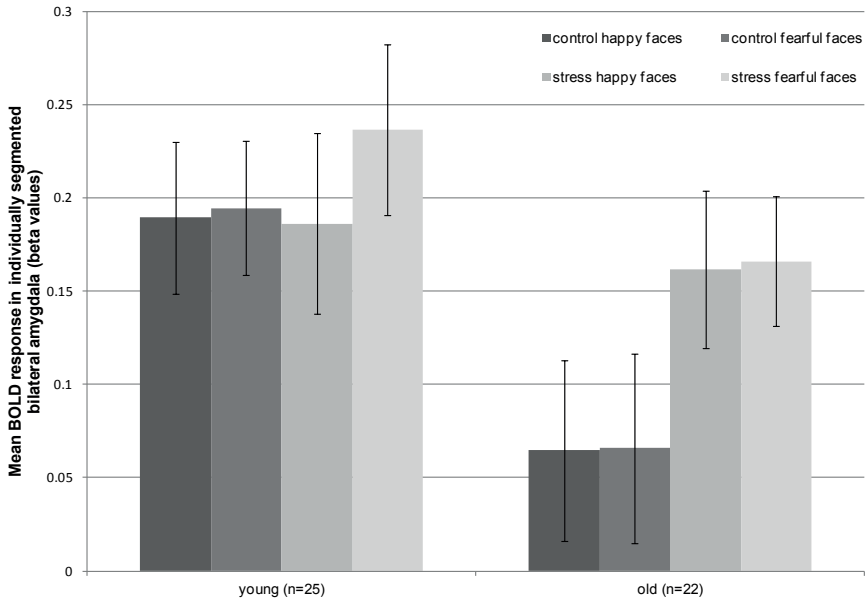
S5.3 Table of fMRI clusters: main effects of task and age.

Effect	BA	Region	Hemi-sphere	Clus-ter size	Peak MNI coordinates			Peak T Value
					x	y	z	
Face processing > Visuomotor control task *(old and young)*	18	Superior occipital gyrus extending into fusiform gyrus	Right + left	51722	16	-96	10	26.00***
					14	-83	-7	24.85***
					-15	-96	6	24.81***
	6, 44	Precentral gyrus extending into the inferior frontal gyrus	Right	1851	48	-2	50	9.53***
					46	4	35	6.36***
					42	13	24	6.35***
	22	Superior temporal gyrus	Right	979	56	-35	12	9.10***
		Superior parietal gyrus	Right	613	27	-50	48	9.03***
	6	Precentral	Left	598	-45	-5	50	7.56***
	21	Superior temporal gyrus	Left	339	-51	-44	11	7.02***
	53	Amygdala	Right	59	21	-6	-16	6.14***
	11	Rectus gyrus	Right + left	370	4	33	-16	5.96**
					-3	39	-16	5.77**
					-3	28	-18	5.54**
		Hippocampus	Left	67	-26	-26	-6	5.89**
		Hippocampus	Right	61	24	-26	-3	5.81**
	53	Amygdala	Left	8	-21	-6	-16	5.30*
	4	Precentral gyrus	Right	5	38	-23	62	5.01*
	38	Temporal pole	Left	1	-36	22	-36	4.95*
		Cerebellum	Left	1	-9	-72	-40	4.93*
Face processing < Visuomotor control task *(old and young)*	40	Supramarginal gyrus	Left	14	-56	-29	33	5.16*
	39	Middle occipital gyrus	Right	3	42	-77	34	4.98*
Stress session > control session *(old and young)*		None	-	-	-	-	-	-
Stress session < control session *(old and young)*		None	-	-	-	-	-	-
Positive effect of age in face processing *(old > young)*		None	-	-	-	-	-	-

S5.3 Table of fMRI clusters: main effects of task and age. (continued)

Effect		Region	Hemi-sphere	Clus-ter size	Peak MNI coordinates			Peak T Value
					x	y	z	
Negative	19	Fusiform gyrus	Right	106	28	-77	-16	5.77**
effect of	18	Calcarine fissure	Left	12	-6	-101	-3	5.25*
age in face	19	Inferior occipital gyrus	Right	11	48	-83	-8	5.14*
processing	19	Fusiform gyrus	Right	10	28	-78	-6	5.13*
(old < young)	19	Inferior occipital gyrus	Right	1	44	-81	-3	4.96*

MNI, Montreal Neurological Institute. BA, Brodmann's area. All analyses are whole brain FWE-corrected: ***p<0.001, ** p<0.01, * p<0.05.

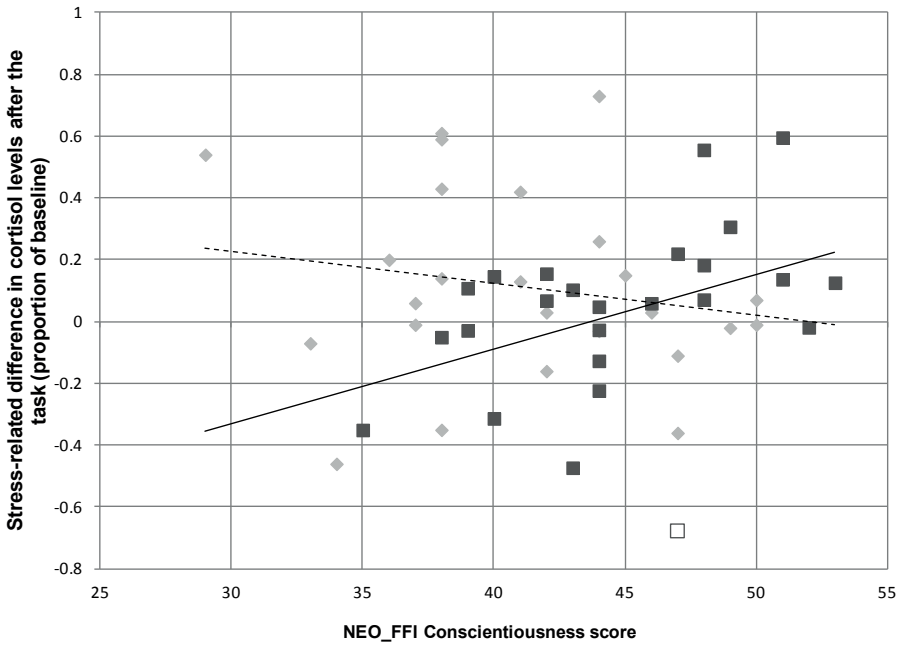


S5.4 Mean amygdala responsivity in response to happy and fearful faces separately in the two sessions per age group.
There are no differences in the group of younger subjects. Within the elderly group, only the response to fearful faces changes significantly under stress compared with the control condition (fearful faces p=0.040; happy faces p=0.104). Amygdala beta values were extracted from the anatomical bilateral amygdala, using individual masks for every subject.

S5.5 Correlations between personality scores and stress–related difference in amygdala responsivity per age group.

	Young (n=25)	Old (n=24)
Altruism	r(23) = -0.233, p = 0.261	r(20) = -0.427, p = 0.037
Conscientiousness	r(23) = -0.042, p = 0.840	r(20) = -0.534, p = 0.007
Extraversion	r(23) = 0.185, p = 0.375	r(20) = -0.294, p = 0.163
Neuroticism	r(23) = -0.096, p = 0.649	r(20) = 0.311, p = 0.139
Openness	r(23) = -0.046, p=0.826	r(20) = 0.000, p = 0.999

Outliers (>2.5) were removed from the analysis.



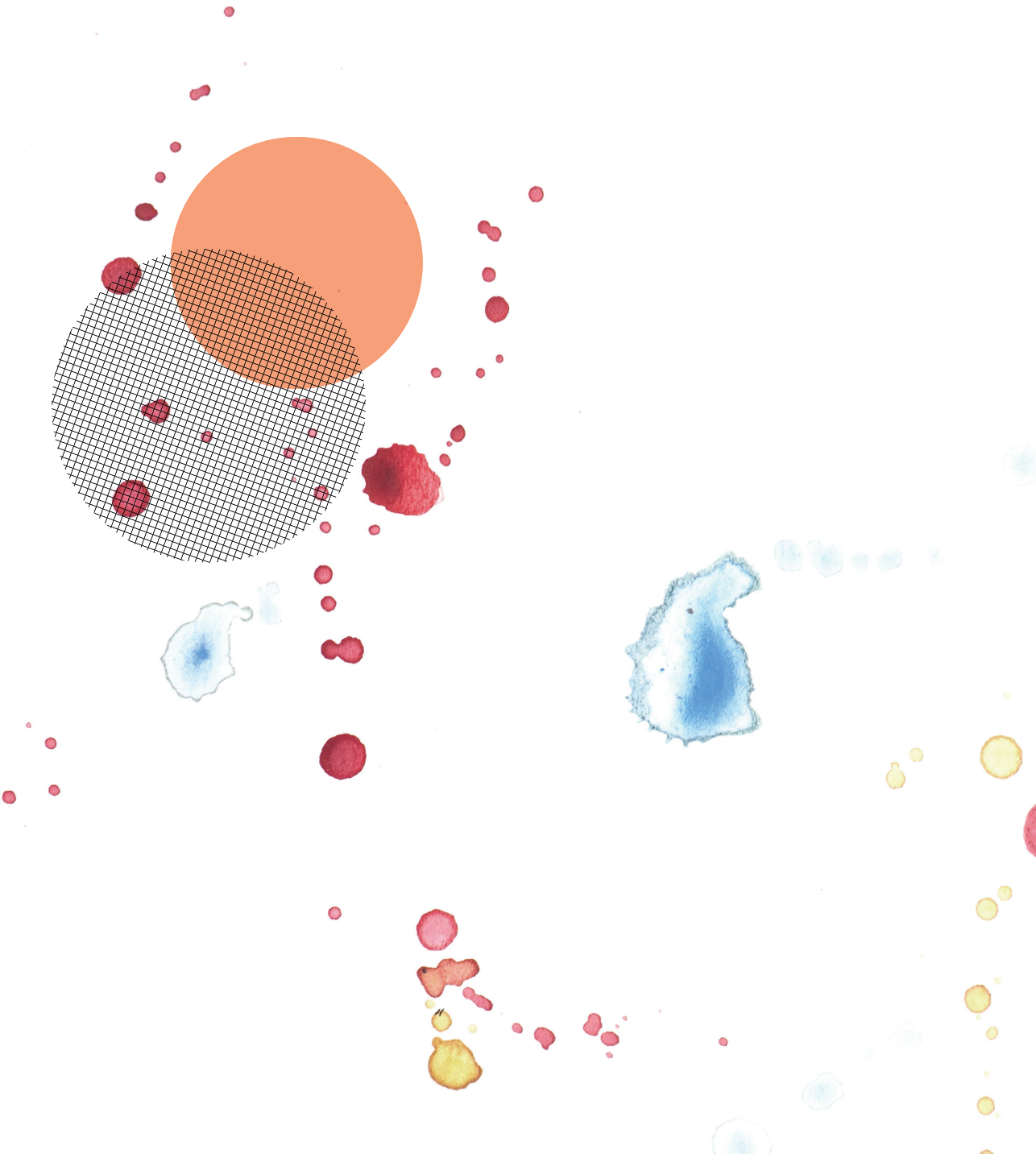
S5.6 Correlation of cortisol levels with conscientiousness

Conscientiousness was negatively correlated (r(22) = 0.534, p=0.007) with the stress-related change in cortisol levels in older adults only. The open square represents an outlier (z-value 2.52) that was excluded from the statistical analysis (r(24) = 0.402, p=0.046 when this subject is included).

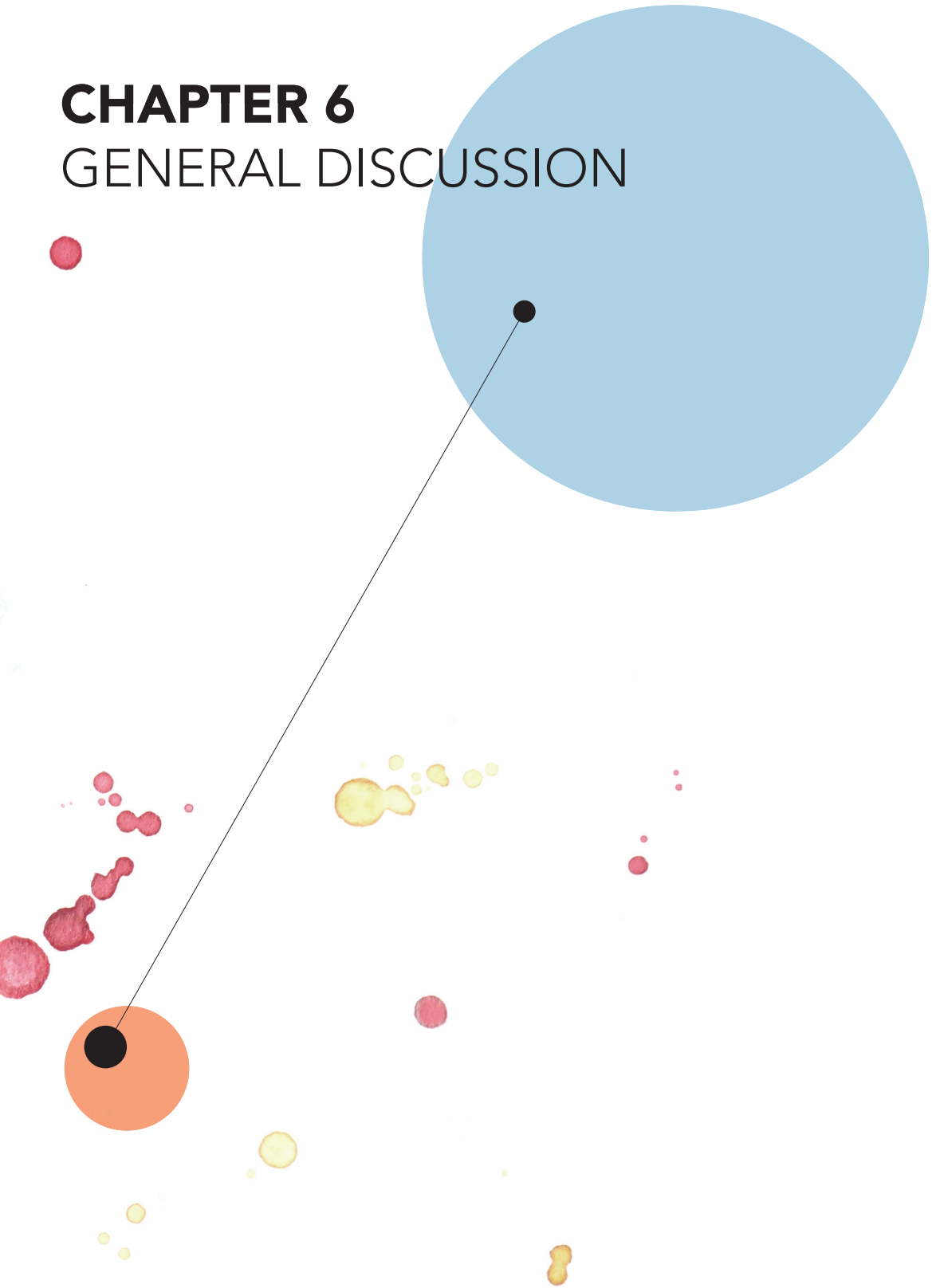
S5.7 Supplemental discussion

Hemodynamic and anatomical differences between younger and older adults are an important factor to take into account when comparing neural activity between these two groups (Samanez-Larkin & D'Esposito, 2008). To circumvent these challenges as much as possible, we employed several strategies. Firstly, we used an adapted spatial normalization procedure: we created a mean template of our subjects (older and younger adults together) and normalized our data to this common template before normalization into standard stereotactic space. Secondly, the extraction of beta values from individually defined

anatomical masks for our main region of interest further limits the potential influence of age-related differences in normalization procedures. Finally, we mainly focused on the interaction effects and within-group effects in our analysis, avoiding direct between-group comparisons as much as possible as they are more prone to potentially confounding baseline differences in hemodynamic responding. Nevertheless, potential age-related differences in for example white matter integrity could still have influenced our findings (I. J. Bennett & Madden, 2014).



CHAPTER 6
GENERAL DISCUSSION



Stress is a very common phenomenon and it most often has a negative effect on our mood and thoughts. Because stress can lead to mental health problems, it is important to understand the mechanisms by which stress influences the functioning of our body and brain. To this end, it is crucial to gain better insight into individual differences in the stress response, as well as distinct effects related to the nature of the stressor.

The work presented in this thesis makes use of different neuroimaging techniques to investigate the effects of both real-life as well as controlled experimentally induced stress on the brain. Firstly, structural brain imaging was used to detect the interactive effect of genetic vulnerability and childhood stress on hippocampal volume, and of different types of childhood adversity on whole brain gray matter structure. In the second part of this thesis we used functional neuroimaging to identify brain regions that are responsive to acute experimentally induced psychological stress in subjects with different personality traits, and investigated differences in stress-related brain responses in healthy aging.

The following general discussion commences with a brief overview of the findings presented in the different chapters. Subsequently, these findings will be discussed in the context of other work by colleagues on this topic. Furthermore, limitations and directions for future research on this topic will be discussed. Finally, the results of this thesis will be related to potential benefits for mental health in society and in clinical practice.

6.1 Summary of findings

PART 1: EFFECTS OF CHILDHOOD STRESS ON BRAIN STRUCTURE

Q1 What are the influences of genetic variance and sex on the neural effects of childhood stress?

In **chapter 2**, we investigated the interactive effect of a common genetic variation, the serotonin transporter-linked polymorphic region (5-HTTLPR), and environmental stressors on the volume of the hippocampus. In addition, we took the potentially moderating factor sex, which is known to affect the prevalence of depression substantially, into account. We found a significant three-way interaction, which was driven by the fact that only male S'-allele carriers exhibited an association between severe childhood adversity and smaller hippocampal volume. Interestingly, there was no main effect of genotype in men, while female S'-allele carriers had smaller hippocampi than L'L' carriers. These results suggest that sex modulates the interactive effect of the 5-HTTLPR genotype and childhood adversity on hippocampal volume. Even though these results require replication, our findings contribute to the understanding of sex differences in the pathophysiology of depression and indicate a mechanistic account of how a specific risk allele and adverse events may increase the vulnerability for depression.

Q2 What is the influence of type of stressor when investigating the effects of childhood stress on the brain?

In **chapter 3**, we explored in a large, well-selected population whether distinct subtypes of childhood adversity, varying in the dimensions of threat and deprivation, could have variable effects on brain structure in healthy young adults. Furthermore, we took potential sex differences into account. Differences in gray matter structure between subjects with a different types of childhood adversity in the past were found in the fusiform gyrus and middle occipital gyrus, where subjects with a history of deprivation showed reduced gray matter compared with subjects with a history of abuse. An interaction between sex and type of adversity was also found. Women showed less gray matter in the visual posterior precuneal region after both subtypes of childhood adversity than controls. Men had less gray matter in the postcentral gyrus after childhood deprivation compared with abuse. Our results suggest that even in a healthy population, subtypes of childhood adversity are related to subtle specific differences in brain structure, which are modulated by sex. Future studies are needed to expand the current findings by examining behavioral consequences of the observed structural differences in even larger, similarly well-selected populations. Finding specific behavioral consequences

of different types of childhood adversity could provide insight into the emergence of distinct neurodevelopmental trajectories, and into potential development of specific psychiatric symptoms and disease in vulnerable individuals.

PART 2: EFFECTS OF ACUTE STRESS ON BRAIN FUNCTION

Q3 What is the influence of personality traits such as neuroticism on the neural stress response?

In **chapter 4**, we studied the relationship between experimentally induced stress, individual differences in neuroticism, and amygdala responsivity using a well-controlled fMRI study design. Here, we demonstrated in a large, homogeneous sample of healthy males that higher trait neuroticism levels predict a greater response of the amygdala to fearful faces, but that this effect depends on the current stressful state of the individual: amygdala responsivity was enhanced only for fearful faces in the stressful as compared to the neutral condition for the more neurotic individuals. These findings indicate enhanced amygdala responsivity in individuals that are at risk for developing stress-related disorders, yet strongly dependent on the stress level of the individual and the valence of the presented stimulus. This effect constitutes one possible neural mechanism for the increase in stress sensitivity and disease risk associated with high levels of trait neuroticism.

Q4 How is the neural stress response affected by healthy aging?

In **chapter 5**, we investigated the neural mechanisms behind the paradox of stable emotional wellbeing during healthy aging, and enhanced vulnerability to stress related disorders in older adults. We found that overall physiological stress reactions were similar across both age-groups, suggesting that our type of stressor had a similar impact across subjects of different ages. Secondly, we replicated earlier findings that positive affect was generally higher in healthy older than younger adults. Importantly, we revealed that healthy, older adults showed increased responsivity of brain regions involved in face and emotion processing while acutely stressed, compared with younger adults. These findings suggest that increased reactivity of affective neural circuitry after acute stress could constitute one mechanism by which emotional wellbeing during healthy aging could rapidly change into heightened vulnerability for affective disorders. Although our study design does not permit statements about causality, this shift in neural activity in the amygdala appeared to be influenced by the personality trait of conscientiousness in the older adults only. Increased understanding of acute stress

responses in healthy aging could help identify elderly at risk for mood and anxiety disorders when confronted with life stressors.

6.2 Integration of findings

In the first part of this thesis, we found that sex is an important moderator of effects of genetic variation and childhood adversity on brain structure. Supporting the evidence for sex specific mechanisms in pathophysiology is important, since there are remarkable differences in the prevalence of different psychiatric disorders, such as a higher prevalence of depression in women and more impulse control disorders in men (Kessler et al., 1993; 2006). Interestingly, sex differences in stress processing can also lead to different outcomes after other health problems, such as stress-related worse recovery from acute myocardial infarction in women than men (Xu et al., 2015). This implies that unraveling sexually dimorphic pathways from stress to maladaptation is essential for understanding the pathophysiology of a variety of stress-related disorders.

The sex differences in brain structure we found in **chapters 2 and 3** could be accounted for by various mechanisms. One apparent mechanism is the influence of gonadal hormones, which could lead to sex differences in neural correlates of childhood stress (Crozier et al., 2014; Young & Korszun, 2010). The serotonergic system is known to be a main target of steroid hormones, which impact the generation and efficacy of serotonergic neurotransmission (Barth, Villringer, & Sacher, 2015). The highest expression of serotonergic neurons is found in the raphe nuclei (midbrain), from which projections exist to various brain regions involved in the stress response, such as frontal cortex, amygdala and hippocampus. Research in nonhuman primates suggests that estrogen impacts serotonergic pathways at various levels, including modification of gene expression (Bethea, Lu, Gundlah, & Streicher, 2002). In sum, evidence suggests that female gonadal hormones can modulate serotonergic effects in the brain, and could hence potentially modulate serotonergic influences on brain development. Male gonadal hormones have more recently been found to also exert effects on serotonergic neurotransmission. Androgen treatment in female-to-male transsexuals increased binding to the serotonin reuptake transporter in amygdala, caudate, putamen, and median raphe nucleus, which also correlated with an increase in testosterone levels, consistent with a potential protective effect of male gonadal hormones (Kranz et al., 2015). In addition to effects on neurotransmission, gonadal hormones can also directly impact the stress response. Salivary cortisol increases in response to stress depend on the menstrual cycle in women, and have been found to be up to twice as high in men as in women (Kudielka et al., 2009). Changes in stress sensitivity have been found to

be related to menstrual cycle-related structural plasticity of the amygdala in humans, illustrating the importance of endogenous fluctuating gonadal hormone levels for the acute stress response (Ossewaarde et al., 2011). Furthermore, estrogen supplementation in perimenopausal women has been found to attenuate blood pressure, glucocorticoid, and catecholamine responses to psychological stress (Komesaroff, Esler, & Sudhir, 1999). With respect to mental health, fluctuation in ovarian hormones are also thought to ultimately lead to HPA axis dysfunction, thereby increasing sensitivity to stress and generating greater vulnerability to depression in perimenopausal women (Gordon et al., 2015). Interestingly, men and women do not only differ in gonadal and stress hormone responses. More complex mechanisms relating to epigenetic differences in sex-biased gene expression most likely also play a role (Jazin & Cahill, 2010). Moreover, different developmental trajectories and therefore 'sensitive' time windows during neural development in boys and girls can also give rise to different consequences after childhood stress (Lenroot et al., 2007; Pechtel, Lyons-Ruth, Anderson, & Teicher, 2014). Finally, different types of stressors may have a dissimilar impact on brain structure in boys and girls. On a behavioral level, sex differences have already been associated with specific vulnerability for different types of stressors. For example, men seem more vulnerable to depression after (sexual) abuse, life events, and instrumental problems, whereas for women, problems relating to interpersonal warmth and relationships have greater impact (Kendler & Gardner, 2014). Additionally, recent evidence suggests that sexual dimorphism of the stress response already starts in the neonatal brain with sex-specific effects of maternal stress on brain development, and is still present in the aging brain, when dynamic hormonal reductions in women are associated with sex-specific aging-related changes in limbic brain regions (Bale & Epperson, 2015). In sum, the findings of this thesis confirm that sex moderates genetic influences (**chapter 2**) as well as stressor-specific effects (**chapter 3**) on brain anatomy in adults in regions that are involved in emotion processing, in line with existing sex differences in stress-related psychiatric disorders.

Crucially, childhood adversity can lead to neural 'damage', but could also lead to adaptive neural effects. These adaptive effects are most likely well represented in our healthy study population, especially in those who have a history of childhood adversity. Some researchers have suggested that early life stress may alter the development of certain brain regions to facilitate survival and reproduction, in line with an evolutionary perspective (Teicher et al., 2016). Notably, adaptive personality traits could mediate this protective effect. For example, a Brazilian study showed that childhood trauma was associated with mostly negative personality traits such as anger, sensitivity and anxiety, and less volition, control, coping and stability (Sudbrack, Manfro, Kuhn, de Carvalho, & Lara, 2015). However, in the same study specifically physical abuse and ne-

glect were positively associated with adaptive personality traits such as more volition and coping as well as less fear, sensitivity and anxiety traits. The negative associations with emotional trauma and positive associations with physical trauma were more pronounced in males than females, suggesting gender specific adaptation mechanisms to specific types of stressors, consistent with the findings in **chapter 3**. Correspondingly, enhanced amygdala responsivity to acute environmental stress, as found in **chapters 4 and 5**, could be adaptive when increased vigilance is necessary for survival (Radley, Morilak, Viau, & Campeau, 2015). Moderate acute stress has indeed been found to positively affect cognitive processes such as attention and working memory, most likely mediated by noradrenergic and catecholaminergic processes (Qin et al., 2012; Sara, 2009). Taken together, stress is mostly associated with negative effects, however from an evolutionary perspective some beneficial effects of stress on brain and behavior seem to exist as well. These beneficial effects are most likely strongly represented in our study population, since we excluded subjects with a history of mental health problems. This implicates that the neural differences we find between subjects with and without childhood adversity in **chapters 2 and 3** could be related to 'neural damage' and vulnerability, as well as to adaptive effects and resilience to childhood stress.

When studying stress vulnerability and resilience, individual differences in terms of genetic variation (**chapter 2**) and personality traits (**chapter 4 and 5**) have an important influence on the stress response. Although we did not investigate this, others have shown that personality traits are most likely an intermediate step between genetic vulnerability and potential disease, such as major depressive disorder (Kendler & Myers, 2010). This is also illustrated by a substantial comorbidity between personality disorders and anxiety disorders, which is largely accounted for by shared genetic susceptibility (Welander-Vatn et al., 2016). Whereas neuroticism is a well-known risk factor for stress-related disorders, the impact of trait conscientiousness on stress responses and stress-related disorders has only recently gained interest (Dahm et al., 2017; Kotov et al., 2010; Servaas et al., 2016; J. R. Swartz, Knodt, Radtke, & Hariri, 2016). The correlation between stress-related amygdala responsivity and conscientiousness we found in **chapter 5** contributes to the emerging awareness that conscientiousness is a personality trait that should not be overlooked when investigating stress sensitivity (Dahm et al., 2017; J. R. Swartz et al., 2016). Remarkably, with respect to aging, trait conscientiousness has also been associated with protective effects against Alzheimer's disease, as well as with reduced general morbidity and mortality in old age, confirming its importance for health maintenance in general (Terracciano et al., 2013; Tolea et al., 2012; Wilson et al., 2007; Wilson, Mendes de Leon, Bienias, Evans, & Bennett, 2004).

In the last chapter of this thesis it is demonstrated that the stress-related change in neural responsivity is not only moderated by individual differences such as genetic predisposition and personality traits, but also appears to be influenced by the aging of the individual. Acute stress selectively enhanced neural activity in visual processing regions as well as the amygdala the elderly, bringing their neural responses to the same level as young adults. These findings are in line with the aging brain model (Cacioppo et al., 2011), suggesting that age-related changes in amygdala activation play a role in mediating an improved emotional state in the healthy older adult, and therefore contributes to our understanding of resilience and emotional aging. Interestingly, effects of stress on the brain resemble those effects that occur in biological aging, since evidence suggests that neuroplastic adaptation to environmental stressors seems similarly impaired in mood disorders as in aging: there is an increased vulnerability for permanent neural damage (McEwen et al., 2015). One important mechanism influencing both stress and neural aging involves the immune system. Preclinical studies show that repeated exposure to stress can lead to changes in microglia, which then results in impaired neuroplasticity and increased risk for depressive behavioral symptoms (Wohleb, Franklin, Iwata, & Duman, 2016). Microglia are the macrophages of the central nervous system and play an important role in neurodegeneration as well as in the pathophysiology of Alzheimer's disease (Piirainen et al., 2017). Childhood adversity has also been found to initiate and sustain inflammatory processes, with persistent elevation of inflammatory biomarkers into adulthood (Nusslock & Miller, 2016). These effects are most likely mediated by molecular processes that occur during stress and that resemble those in aging, such as altered gene expression through changes in epigenetic mechanisms and altered telomere length, a marker of cellular aging (McEwen et al., 2015; Ridout et al., 2017). In the light of these findings, it is not surprising that stress in aging has also been described as 'adding fuel to the fire' (Prenderville et al., 2015). In this argumentation, childhood adversity may then be seen as the 'firelighters', starting a cascade of molecular and cellular changes that can eventually lead to impaired neural plasticity, altered behavioral traits as measured in personality characteristics and eventually mental disease (fig. 6.1). This cascade is additionally influenced by factors such as sex differences, specific impact related to the type of environmental stressors and different 'windows' of vulnerability across the life cycle. Considering the many systems involved, it is then not surprising that the ultimate behavioral consequences are difficult to trace back to a simple genetic variance or brain region. In contrast, the studies described in this thesis contribute evidence that studying interactions between the variety of factors in this cascade can provide us with a better mechanistic understanding of how vulnerability can eventually lead to maladaptation and disease.

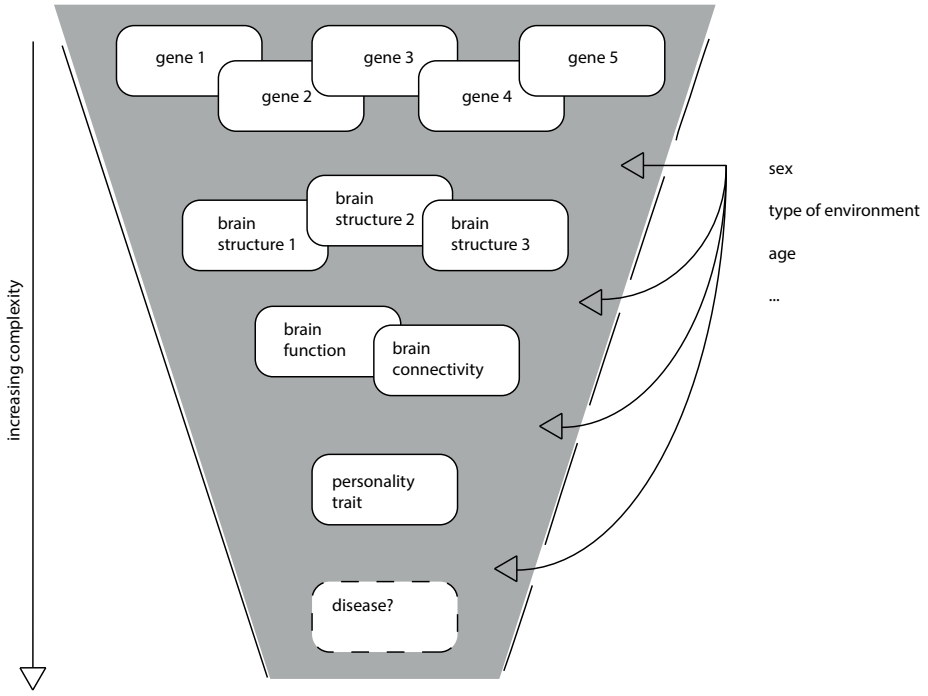


Figure 6.1 Adapted model of the endophenotype concept in psychiatry.
Simple but often multiple genetic variations cause differences in brain anatomy, which lead to differences in brain function. These changes finally result in complex behavioral differences. Factors such as sex, age and environmental influences interact with each of these levels, as presented in this thesis. Adapted from (Franke et al., 2009).

6.3 Limitations and future directions

Limitations of the work presented in this thesis include the type of genetic analysis, methods to assess a history of childhood adversity and the selection of the study populations. These limitations will be discussed in this section, and potential strategies to cope with these limitations in the future will be provided.

One clear limitation of the genetic analysis presented in **chapter 2** is the absence of a replication sample. Since effects of genetic variation on brain anatomy are small, it has become a standard procedure to repeat the genetic analysis in an independent sample in order to reduce the chance of false positive findings (Balding, 2006). In addition, other challenges faced by research on genetic moderation of stress effects faces include reliably measuring the environment and neural phenotypes, the absence of

detailed mechanisms and the influence of neural development (Bogdan, Pagliaccio, Baranger, & Hariri, 2016).

Moreover, with respect to the serotonin transporter gene polymorphism, a recent collaborative meta-analysis concluded that there is no broadly generalizable interaction between 5-HTTLPR, stress and depression (Culverhouse, Saccone, Horton, & Ma, 2017). Importantly, in line with the conclusion from the previous paragraph, one explanation for this last finding is that depression as an outcome measure is too heterogeneous. Intermediate phenotypes, such as changes in brain structure or function, could be more sensitive to subtle genetic differences (Franke et al., 2009). Indeed, another study confirmed our finding in **chapter 2** that hippocampal structure is influenced by 5-HTTLPR x stress interactions (Rabl et al., 2014). Behavioral phenotypes, such as sensory processing sensitivity, are other intermediate phenotypes that can contribute to our understanding of 5-HTTLPR related stress vulnerability (Homberg, Schubert, Asan, & Aron, 2016). In sum, future studies should not only aim at replicating findings of genetic vulnerability in a second independent sample, but also focus more on intermediate anatomical, physiological and behavioral phenotypes, and integrate factors such as age and sex, as described in figure 6.1.

Of note, the candidate gene analysis we performed in **chapter 2** is only one method to investigate genetic variation in stress sensitivity. Other available methods to study genetic variation include family, twin and adoption studies, genome-wide association studies (GWAS) and pathway analyses (Kendler, 2013; Sullivan, Daly, & O'Donovan, 2012). Although these methods are more demanding in terms of the selection of the study population (they either require family members to participate or a very large number of subjects to be included), they also have some important advantages that make them promising for future research. For example, in GWAS many genes can be tested at once, circumventing potential selection bias and taking into account the polygenic nature of complex behavioral traits (Sullivan et al., 2012).

Secondly, the assessment of childhood severity in **chapter 2 and 3** was done with an adapted version of the 'List of Threatening Life Events', a self-report questionnaire (Brugha et al., 1985). While this questionnaire provides information on a wide range of moderate to severe life stressors, it does not document the exact timing, duration nor the frequency of the stressor. In addition, retrospective questionnaire might introduce uncertainty in the measurement, since it can be questioned to which extent retrospective reports of childhood maltreatment reflect reality: they might be biased by the subjective experience of the victim and the current affective state. On the other hand, similar to medical history taking, adverse childhood experiences will frequently

be assessed using a retrospective approach in practice. Another frequently used questionnaire, the Childhood Trauma Questionnaire, has similar limitations (Bernstein et al., 2003). In the light of these limitations, a new instrument named the 'Maltreatment and Abuse Chronology of Exposure' (MACE) was recently developed, incorporating type and timing of exposure (Teicher & Parigger, 2015). The first experiences with this questionnaire seem promising, with high test-retest reliability over time and, despite its retrospective assessment, very little evidence for negative attribution bias. Therefore, in future studies the MACE should be considered as an instrument to assess adverse childhood experiences.

A third limitation involves the selection of the study population. Here, two characteristics of the subjects are important: sex and age. With respect to sex differences, the findings from **chapter 2 and 3** confirm the importance of sexual dimorphism of the association between stress and brain structure. Nevertheless, in **chapters 4 and 5** we investigated stress effects on brain function in males only. Although this seems contradicting, one important reason for including males only is that in these exploratory studies we aimed to keep the sample as homogeneous as possible in order to avoid variance caused by other factors (such as sex) to influence the effects we found. Critically, this implicates that our findings in **chapters 4 and 5** are not easily generalizable to female populations (Schwabe et al., 2013) and thus need to be replicated in a mixed sample of males and females. Furthermore, in the light of the age-related differences in neural stress responsivity we find in **chapter 5**, the studies performed in the relatively young populations of **chapter 2, 3 and 4** should be replicated in a sample with a larger, preferably continuous age range, including older adults (Ioannidis, 2017). Finally, the samples we used all consist of healthy and high-performing participants, and therefore most likely represent a relatively resilient subsample of the population. Future studies should also include more vulnerable populations, preferably in a continuum of resilience to vulnerability, in order to improve our understanding of the transition from adaptation to maladaptation to stress. Based on our findings in **chapter 4**, trait neuroticism could potentially provide an interesting phenotype when assembling a heterogeneous study population to investigate neural differences in stress responsivity. This is only feasible when including much larger sample sizes. In addition, behavioral correlates of the neural differences we found could provide more information on the pathophysiological pathways to mental disease.

To summarize, it remains to be investigated how our findings translate to a more heterogeneous population including different sexes, ages, educational attainments, and across a continuum from health to disease.

6.4 Implications for mental health

The research that is presented in this thesis was exclusively performed in healthy individuals. Studying the neural stress response in this population can be very valuable for society and clinic. Here, I will briefly outline the recent progress that has been made in using 'basic' neuroimaging methods as described in this thesis to aid policy makers and clinicians in the prevention of disease, prediction of disease courses and finally, in guiding treatment decisions in patients.

From a public health point of view, prospectively identifying characteristics or even biomarkers of resilience to stress is of major importance. For example, identifying susceptible versus less susceptible individuals can aid in targeting interventions after a major stressor (Walker, Pfingst, Carnevali, Sgoifo, & Nalivaiko, 2017). In addition, if we understand the mechanisms that underlie resilience to stress, resilience-enhancing interventions could be developed. For example, it has been suggested that individuals who seem resilient to childhood adversity are not 'unaffected', but rather 'compensating' (Teicher et al., 2016). Finding the underlying compensatory mechanisms could help in developing tools to prevent mental disease to occur after childhood trauma and other stressful life events. Potential resilience-enhancing tools could for example consist of top-down behavioral interventions that could 're-open' and intervene in windows of vulnerability, even later in life (Karatoreos & McEwen, 2013). With respect to childhood deprivation, effects of resilience-enhancing strategies have been found for IQ, language development, reward learning, neural function, and white matter volume and integrity (McLaughlin, Sheridan, & Nelson, 2017). Other behavioral interventions to promote stress resilience could consist of lifestyle adaptations to slow aging mechanisms in the brain, such as healthy diets and exercise (Stranahan & Mattson, 2012). Considering our findings of sex as a moderator of childhood stress effects in the brain in **chapter 2 and 3**, future studies should also explore the possibility of developing more targeted gender-specific resilience-enhancing strategies.

Concerning resilience in aging, there is evidence suggesting that increasing resilience in older adults might have very strong effects on self-rated successful aging, as strong as effects of enhancing physical functioning (Jeste et al., 2013). A recent review suggests that emotion regulation and social support may be two primary factors that contribute to resilience to chronic stress in older adults, partly by modulating HPA activity (Gaffey, Bergeman, Clark, & Wirth, 2016). These factors could be potential mechanisms for resilience-promoting interventions for older adults in the future. In **chapter 5** we investigated effects of acute stress on neural processing in healthy, and most likely relatively resilient, older adults. While older adults showed less responsivity of face

processing regions and the amygdala than younger subjects in a neutral condition, stress enhanced brain responses to similar levels as in young adults. This implies that acute stress may affect neural mechanisms of resilience more strongly in older than young adults, and therefore should be taken into consideration when investigating resilience-enhancing strategies in aging.

Findings from brain imaging can also provide a clinician with diagnostic or prognostic information. For example, increased structural brain aging has been associated with a greater risk of death and worse physical and cognitive health in older adults (J. H. Cole, Ritchie, Bastin, & Hernández, 2017). With respect to amygdala responsivity, as investigated in **chapters 4 and 5**, threat-related amygdala responsivity in young adults has been found to predict vulnerability to stress in the future (J. R. Swartz et al., 2015). In older adults, neural responses to emotional faces have been associated with suicidal behavior in this population (Vanyukov et al., 2015). Therefore, gaining a better understanding of the influence of acute stress on amygdala responsivity to emotional stimuli could potentially have specific implications for clinical decision making in different kinds of populations. Another study found that the future disease course of major depressive disorder can be more reliably predicted when using neuroimaging data than when using clinical measures only, confirming the importance of integrating information from different levels of the endophenotype model depicted in figure 6.1 (Schmaal et al., 2014). However, for these techniques to be useful in clinical practice, new methods need to be developed that are reliable at the single patient level, and not at the group level only.

Finally, neuroimaging data can be used to classify individuals into subpopulations that differ in susceptibility to psychiatric disease, prognosis or treatment response: the field of precision medicine (Collins & Varmus, 2015). A more targeted approach to disease and treatment is of major interest to the field of psychiatry, where disorders are heterogeneous, many options for treatment exist, and response rates can be low. Precision medicine aims to detect predictors or biomarkers that can identify patients who will or will not respond to a certain type of treatment, and is already used in fields such as oncology where molecular diagnoses help guide treatment choices (Insel & Cuthbert, 2015). The RDoc domains described in box 1.2 not only provide an excellent framework to help identify new targets for treatment development, but can also help to detect subgroups for treatment selection and therefore precision medicine in psychiatry (Insel & Cuthbert, 2015). Neuroimaging data can play an important role in the development of these predictors. For example, several neuroimaging predictors have been identified for antidepressant treatment response, such as pretreatment amygdala and ACC activity (M. L. Phillips et al., 2015). Understanding the dynamics of

amygdala responsivity under different conditions and in different subjects, as investigated in **chapters 4 and 5**, is therefore important with respect to these developments. Additionally, neurophysiological subtypes of depression have been found using resting-state connectivity in large samples of patients, that predicted responsiveness to transcranial magnetic stimulation therapy (Drysdale et al., 2016). Advances have also been made in the use of pattern recognition and aggregated neuroimaging data when developing predictive models for neurological and psychiatric disease (Woo, Chang, Lindquist, & Wager, 2017). Crucially, regarding **chapter 2 and 3**, childhood adversity has been found to strongly influence the phenotypic expression of psychopathology and could be used to differentiate different types of syndromes, crossing current diagnostic boundaries, to develop better treatment strategies (Teicher & Samson, 2013). Our findings in these chapters additionally suggest that sex can be an important moderator of these phenotypic expressions.

Taken together, although these methods are not yet reliable on a single patient level, it is likely that predictors of disease course and treatment response in the future will be based on a combination of biomarkers, integrating genetic information with neural anatomy, function and psychological factors such as personality traits (Insel & Cuthbert, 2015; M. L. Phillips et al., 2015) (fig. 6.2). The findings described in this thesis suggest that in addition to and in interaction with these factors, individual differences in neural stress sensitivity and resilience are important biomarkers to integrate within this model.

6.5 Concluding remarks

"A strong man's head is full of scars" – African proverb.

As described in this thesis, stress has a significant and long-lasting impact on our brains and behavior. The intensity and the extent of the impact depend on many factors. Genetic susceptibility, personality traits, sex, age and the nature of the stressful experience all shape our brains and future behavior. The current thesis reveals several neural mechanisms for these influences, including hippocampal volumes differences, alterations in amygdala responsivity to emotional facial expressions and gray matter differences in various brain regions. Improving the understanding of these mechanisms that underlie our vulnerability or resilience to stressors that occur across the life span is the basis for future development of resilience-enhancing strategies for vulnerable individuals.

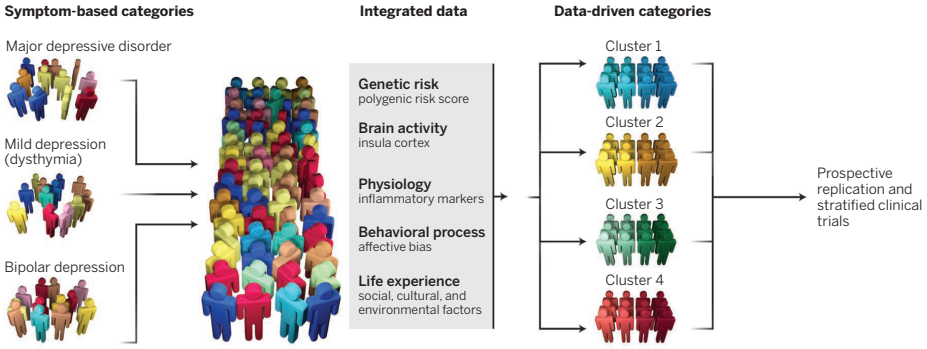
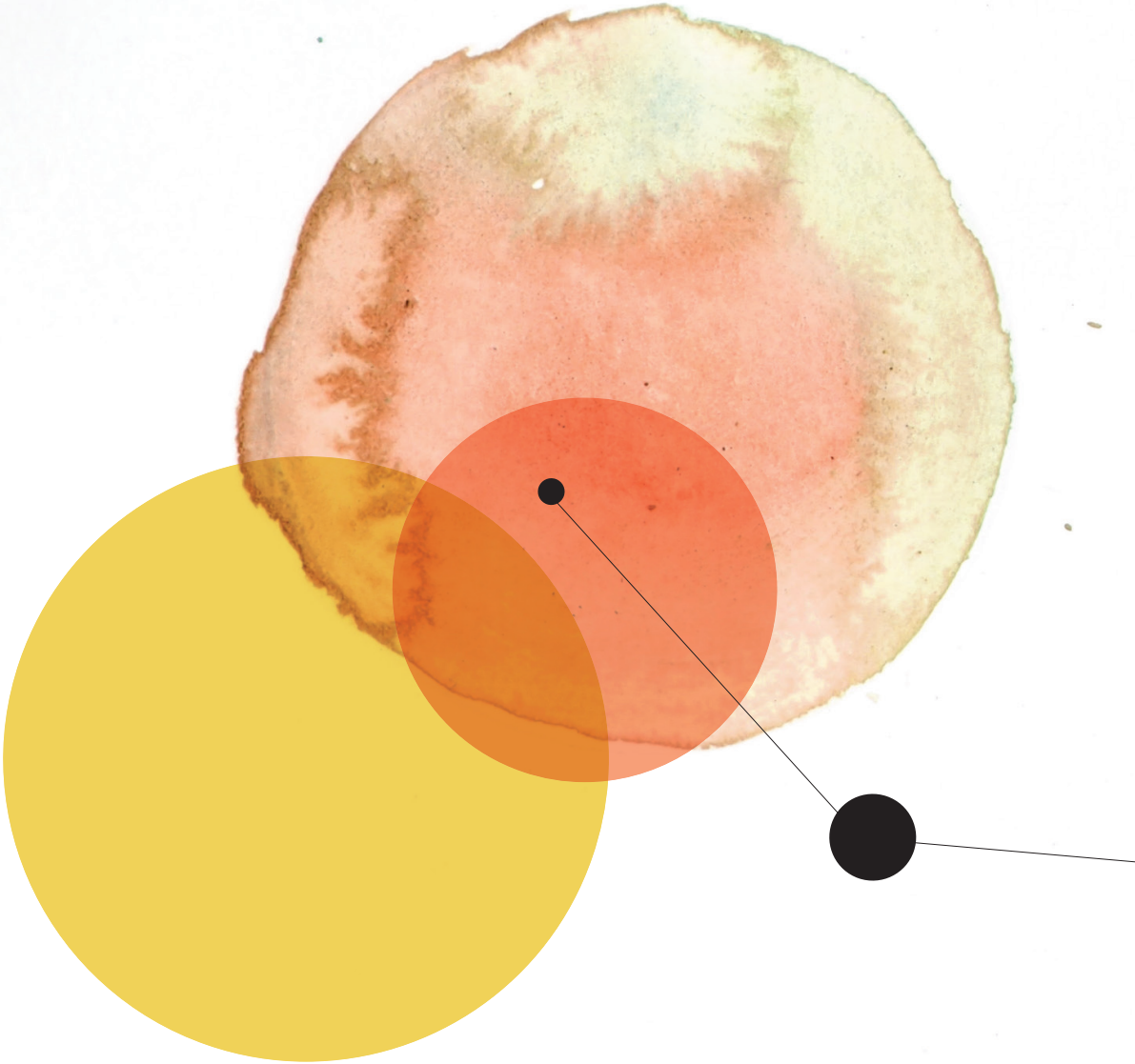


Figure 6.2 Example of how integration of information from different biomarkers, including neuroimaging data, can help identify subgroups of patients that may profit similarly from a certain type of treatment. From (Insel & Cuthbert, 2015). Reprinted with permission from AAAS.



APPENDIX

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Nederlandse samenvatting

Iedereen weet hoe het voelt om gestrest te zijn: toen je klein was en je kleine broertje er vandoor ging met je favoriete speelgoedauto of misschien onlangs nog toen je in de file stond op weg naar een belangrijke afspraak. Door het leven heen maken we vele stressoren mee, variërend van te laat komen voor een afspraak tot meer ernstige stressoren, zoals het verliezen van je baan, een scheiding, gezondheidsproblemen of het verliezen van een dierbare. Over het algemeen is onze reactie op stress adaptief, wat wil zeggen dat onze reactie gericht is op aanpassing, en wordt al snel een nieuw evenwicht gevonden in relatie tot de stressor (***homeostase***). Wanneer de stress ernstig is of lang aanhoudt kunnen we echter uit balans raken: het aanpassen lukt dan niet meer zo goed (***allostase***). In sommige gevallen kan stress zelfs een belangrijke rol spelen in het ontstaan van gezondheidsproblemen, zoals angststoornissen of depressie. Om die reden is het belangrijk om beter te begrijpen hoe stress onze hersenen en ons gedrag beïnvloedt.

Niet iedereen reageert op dezelfde wijze op stress. Waar sommige mensen door een onverwachte file al behoorlijk uit balans kunnen raken, hebben anderen nauwelijks last van een stressvolle ervaring. Een van de redenen hiervoor is dat dezelfde stressor een verschillende impact kan hebben op verschillende mensen. Onderzoek heeft uitgewezen dat de stressrespons bijvoorbeeld op verschillende niveaus wordt beïnvloed door sekseverschillen. Naast de rol van genen en omgevingsfactoren, effecten van geslachtshormonen en stresshormonen, verschillen in hersenanatomie en hersenfuncties, lijken er ook verschillen te bestaan in de impact van specifieke stressoren tussen mannen en vrouwen. Stress gerelateerde psychiatrische aandoeningen, zoals angststoornissen en depressie, komen dan vaker voor bij vrouwen dan bij mannen. Leeftijd is een andere factor die van invloed is op onze stressrespons. Kinderen en ouderen zijn kwetsbaarder voor stressvolle invloeden van buitenaf, waarschijnlijk omdat hun hersenen in deze periodes veel veranderingen ondergaan. Ouderen hebben daarnaast ook de gevolgen van chronische stress met de jaren “opgestapeld” en minder mogelijkheden tot herstel van nieuwe gevolgen van stress. Daarentegen zijn er ook aanwijzingen dat gezonde veroudering gepaard gaat met betere weerbaarheid voor stress: gezonde ouderen zijn over het algemeen beter in het reguleren van hun emoties dan jongeren. Tenslotte spelen ook individuele verschillen een rol. Genetische verschillen kunnen leiden tot verschillen in hersenstructuur en hersenfunctie. Dit speelt vervolgens een rol in de ontwikkeling van verschillende persoonlijkheidskenmerken, welke ook meespelen in het ontstaan van een angststoornis of depressie. Dit alles maakt dat verschillende mensen op dezelfde stressor toch anders kunnen reageren.

Een andere reden voor verschillen in impact van stress is dat de ene stressvolle ervaring de andere niet is. De duur, hoeveelheid, ernst en ook het type stressor bepaalt mede hoe iemand op de stress reageert. Zo is bijvoorbeeld gebleken dat een kindertijd waarin armoede en verwaarlozing een belangrijke rol spelen aanleiding kan geven tot onderontwikkeling van hersengebieden die sensorische informatie verwerken. Blootstelling aan bedreigende situaties in de kindertijd kan daarentegen leiden tot toegenomen activiteit in hersengebieden die betrokken zijn bij het verwerken van emoties, passende bij bijvoorbeeld toegenomen waakzaamheid.

Effecten van stress kunnen op verschillende manieren worden onderzocht. Levensechte stress wordt meestal onderzocht op retrospectieve wijze: vragenlijsten worden afgenomen om traumatische ervaringen gedurende het leven in kaart te brengen. Stress kan echter ook op een gecontroleerde wijze worden toegediend aan proefpersonen in een experimentele setting, bijvoorbeeld in de vorm van een speech of hoofdrekenen voor een kritisch publiek (de bekende Trier Social Stress Task). Ook zijn er speciale methoden ontwikkeld om stress toe te dienen in een MRI-scanner, bijvoorbeeld door het laten zien van gewelddadige filmfragmenten, zodat tegelijkertijd hersenactiviteit kan worden gemeten.

Samenvattend zijn dus vele factoren van invloed op de stress respons en is het belangrijk om deze factoren beter te leren begrijpen, onder andere omdat stress een belangrijke rol speelt in het ontstaan van stress gerelateerde psychiatrische aandoeningen. Dit heeft geleid tot de volgende **onderzoeksvragen** die in dit proefschrift behandeld zullen worden:

1 Wat zijn de invloeden van genetische variatie en sekse op het effect van stress in de kindertijd op de hersenen?
2 Wat is de invloed van het type stressor op de effecten van stress in de kindertijd op de hersenen?
3 Wat is de invloed van persoonlijkheidskenmerken zoals neuroticisme op de neurale stress respons?
4 Hoe wordt de neurale stress respons beïnvloed door gezonde veroudering?

In het **tweede hoofdstuk** van dit proefschrift hebben we onderzoek gedaan naar de invloeden van genetische variatie en sekse op het effect van stress in de kindertijd op hippocampusvolume. De hippocampus is een hersengebied wat betrokken is bij onder andere informatieverwerking, geheugen en het verwerken van emoties. De genetische

variatie waar we naar hebben gekeken is die van het serotonine transporter gen polymorfisme (5-HTTLPR). Deze genetische variatie geeft waarschijnlijk een grotere kans op het krijgen van depressieve symptomen, met name na het doormaken van stressvolle gebeurtenissen. In eerdere soortgelijke onderzoeken is de mogelijke invloed van sekse niet eerder meegenomen. We hebben deze driewegsinteractie tussen 5-HTTLPR, stressvolle gebeurtenissen en sekse onderzocht met behulp van anatomische MRI-scans van de hersenen bij 357 gezonde proefpersonen. Een interactief effect op hippocampusvolume werd gevonden tussen ernstige stressvolle gebeurtenissen in de kindertijd met genotype en sekse ($p = 0.010$), alsmede een interactie tussen genotype en ernstige stressvolle gebeurtenissen in de kindertijd ($p = 0.007$) bij mannen. Post hoc tests lieten zien dat alleen mannelijke S'-allel dragers met ernstige stressvolle gebeurtenissen in de kindertijd kleinere hippocampi hadden ($p = 0.002$). Er was geen hoofdeffect van genotype bij mannen, maar vrouwelijke S'-allel dragers hadden wel kleinere hippocampi dan vrouwen met het L'L' genotype ($p = 0.023$). Onze resultaten laten zien dat sekse de tweewegsinteractie tussen het 5-HTTLPR genotype en stressvolle gebeurtenissen in de kindertijd op hippocampusvolume beïnvloedt. Deze informatie verheldert de invloed van sekseverschillen in samenhang met genetische kwetsbaarheid en stressvolle gebeurtenissen in de kindertijd op hersenstructuur.

Hoofdstuk drie van dit proefschrift beschrijft een onderzoek dat we hebben gedaan naar het effect van type stressor op hersenstructuur in een grote groep gezonde proefpersonen. Daarbij hadden we speciale aandacht voor mogelijke sekseverschillen in deze effecten, omdat er naast prevalentieverschillen in stress gerelateerde aandoeningen bij mannen en vrouwen ook sekseverschillen bestaan in hersenontwikkeling tijdens de kindertijd en adolescentie. Uit een grote databank van proefpersonen selecteerden we proefpersonen met bepaalde specifieke ervaringen in de kindertijd, waarbij we drie groepen maakten: proefpersonen met mishandeling of misbruik in de kindertijd (n=127), proefpersonen met verlieservaringen of armoede in de kindertijd (n=126) en een controlegroep zonder traumatische ervaringen in de kindertijd (n=129). De groepen waren vergelijkbaar wat betreft leeftijd, geslacht en opleidingsniveau. Vervolgens hebben we gekeken naar grijze stof verschillen op MRI-scans van de hersenen tussen deze drie groepen. Verschillen tussen proefpersonen met verschillende soorten traumatische ervaringen werden gevonden in de gyrus fusiformis en in een occipitaal gebied, waar proefpersonen met alleen verlieservaringen of armoede in de kindertijd minder grijze stof volume hadden dan proefpersonen met alleen mishandeling of misbruik in de kindertijd. Interacties met sekse werden ook gevonden. Vrouwen met traumatische ervaringen in de kindertijd hadden minder grijze stof volume in het posterieure gedeelte van de precuneus dan vrouwen zonder traumatische ervaringen in de kindertijd. Mannen met alleen verlieservaringen of armoede in de kindertijd

hadden minder grijze stof volume in de gyrus postcentralis dan mannen met alleen mishandeling of misbruik in de kindertijd. Deze resultaten tonen aan dat subtiele grijze stof verschillen gerelateerd aan specifieke ervaringen in de kindertijd zichtbaar zijn bij gezonde mensen. Deze kennis draagt bij aan een beter begrip van de gevolgen van traumatische ervaringen in de kindertijd op onze (hersen)ontwikkeling en gedrag.

In het **vierde hoofdstuk** hebben we onderzocht hoe individuele verschillen in mate van neuroticisme, een persoonlijkheidskenmerk dat sterk samenhangt met angststoornissen en depressie, kunnen leiden tot verschillen in de neurale stress respons. Hierbij waren we vooral geïnteresseerd in de reactiviteit van de amygdala, een hersengebied dat onder andere hyperreactief is bij patiënten met angst- en stemmingsstoornissen. Bij 120 gezonde jonge mannen hebben we de reactiviteit van de amygdala gemeten tijdens het laten zien van blije en bange gezichten in de MRI-scanner. Dit hebben we bij alle proefpersonen tweemaal gedaan in twee aparte sessies, waarbij tijdens één sessie stress werd toegediend aan de proefpersonen door het laten zien van gewelddadige filmfragmenten. In de andere sessie werden neutrale filmfragmenten vertoond. Bij dit onderzoek zagen we dat gezonde proefpersonen die hoog scoorden op een neuroticisme vragenlijst een grotere toename van amygdala reactiviteit hadden bij het zien van bange gezichten dan proefpersonen die laag scoorden op neuroticisme. Dit trad echter alleen op wanneer zij ook stress toegediend kregen. Deze bevinding geeft nieuwe inzichten binnen de complexiteit van het ontstaan van angst- en stemmingsstoornissen.

Hoofdstuk vijf van dit proefschrift geeft tenslotte een studie weer die we deden naar de invloed van gezonde veroudering op de neurale stress respons, waarbij we opnieuw met name geïnteresseerd waren in de amygdala respons. Voor deze vraag gebruikten we dezelfde onderzoeksopzet als in hoofdstuk vier, echter vergeleken we nu twee groepen mannen die verschilden in leeftijd: een groep van 18-30 jaar (n=25) en een groep van 60-75 jaar (n=25). De groepen waren vergelijkbaar in wat betreft opleidingsniveau en angstgevoeligheid. Hierbij vonden we dat door acute stress een toename van reactiviteit optrad in de gyrus fusiformis bij ouderen, maar niet bij jongeren. In de amygdala vonden we eenzelfde patroon van selectieve toename van reactiviteit door stress bij ouderen. Deze stress gerelateerde toename van activiteit bij ouderen correleerde met de mate van consciëntieusheid (gewetensvol en doelgericht zijn) als persoonlijkheidskenmerk. Deze bevindingen geven aanwijzingen voor een mogelijk mechanisme waardoor beter emotioneel welzijn bij gezonde ouderen door acute stress kan veranderen in toename van kwetsbaarheid voor stressoren.

Samenvattend laten deze studies zien dat vele factoren het effect van stress op onze hersenen beïnvloeden: genetische verschillen en persoonlijkheidskenmerken, sekseverschillen en leeftijdsverschillen interacteren op complexe wijze met elkaar en geven daarmee aanleiding tot toegenomen kwetsbaarheid of weerbaarheid van de hersenen voor de invloeden van stress. Het beter leren begrijpen van deze mechanismen kan in de toekomst bijdragen aan een verbeterde emotionele gezondheid, bijvoorbeeld door het ontwikkelen en toepassen van preventieve maatregelen bij mensen met verhoogde (neurale) kwetsbaarheid voor stress. Binnen de psychiatrie zou nieuwe kennis over de neurale stress respons in de toekomst wellicht kunnen helpen om bij patiënten onderlinge verschillen in het beloop van ziekte en behandelrespons te kunnen voorspellen.

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List of publications

Peer reviewed publications

Everaerd D., Klumpers F., Oude Voshaar R., Fernández G., Tendolkar I. (2017) Acute stress enhances emotional face processing in the aging brain. ***Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2***(7), 591-598. http://dx.doi.org/10.1016/j.bpsc.2017.05.001.

Young C.B., Raz G., **Everaerd D.**, Beckmann C.F., Tendolkar I., Hendler T., Fernández G., Hermans E.J. (2017). Dynamic shifts in large-scale brain network balance as a function of arousal. ***Journal of Neuroscience***, ***37***(2), 281-290. http://doi: 10.1523/JNEUROSCI.1759-16.2017.

Everaerd D., Klumpers F., van Wingen G., Tendolkar I., Fernández G. (2016). Author's response to commentary 'Depressive symptomatology should be systematically controlled for in neuroticism research'. ***Neuroimage, 125***, 1101-2. http://doi: 10.1016/j.neuroimage.2015.08.039.

Everaerd D., Klumpers F., Zwiers M., Guadalupe T., Franke B., van Oostrom I., Schene A., Fernández G., Tendolkar I. (2016). Childhood abuse and deprivation are associated with distinct sex-dependent differences in brain morphology. ***Neuropsychopharmacology, 41***(7), 1716-23. http://doi: 10.1038/npp.2015.344.

Henckens M.J., Klumpers F., **Everaerd D.**, Kooijman S.C., van Wingen G.A., Fernández G. (2015). Inter-individual differences in stress sensitivity: Basal and stress-induced cortisol levels differentially predict neural vigilance processing under stress. ***Social Cognitive and Affective Neuroscience, 11***(4), 663-73. Htt://doi: 10.1093/scan/nsv149.

Everaerd D., Klumpers F., van Wingen G., Tendolkar I. (2017), Fernández G. (2015). Association between neuroticism and amygdala responsivity emerges under stressful conditions. ***Neuroimage***, ***112***, 218-224. http://doi: 10.1016/j.neuroimage.2015.03.014.

Klumpers F., Kroes M.C., Heitland I., **Everaerd D.**, Akkermans S.E., Oosting R.S., van Wingen G., Franke B., Kenemans J.L., Fernández G., Baas J.M. (2014). Dorsomedial Prefrontal Cortex Mediates the Impact of Serotonin Transporter Linked Polymorphic Region Genotype on Anticipatory Threat Reactions. ***Biological Psychiatry, 78***(8), 582-9. http://doi: 10.1016/j.biopsych.2014.07.034.

Everaerd D., Gerritsen L., Rijpkema M., Frodl T., van Oostrom I., Franke B., Fernández G., Tendolkar I. (2012). Sex modulates the interactive effect of the serotonin transporter gene polymorphism and childhood adversity on hippocampal volume. ***Neuropsychopharmacology, 37***(8), 1848-55. http://doi: 10.1038/npp.2012.32.

Rijpkema M., **Everaerd D.**, van der Pol C., Franke B., Tendolkar I., Fernández G. (2012). Normal sexual dimorphism in the human basal ganglia. ***Human Brain Mapping, 33***(5), 1246-52. http://doi: 10.1002/hbm.21283.

(International) conferences

2017 Workshop 'Neuroimaging for psychiatrists' at the annual meeting of the Dutch Psychiatric Association, Maastricht, the Netherlands.

2015 Oral presentation at the Society for Biological Psychiatry, Annual Scientific Meeting, Toronto, Canada.

2013 Poster presentation at the Society for Neuroscience, Annual Meeting, San Diego, USA.

2012 Oral presentation at the Dutch Endo-Neuro-Psycho Meeting, Lunteren, the Netherlands.

Photo by Florent Schmaltz

Curriculum Vitae

Daphne was born on the 7th of January 1984 in Roosendaal, the Netherlands. After her high school graduation in 2002 at the Gymnasium Juvenaat in Bergen op Zoom, she went to study medicine in Nijmegen. During her studies, she spent five months at Université Claude Bernard in Lyon, France and three months at Ghent University in Belgium. At both universities she followed courses at the faculty of medicine and completed clinical internships through the Erasmus exchange program. During medical school, she completed the two-year extracurricular Honours Program and was an active member of different student organizations. In 2006-2007 she completed her research internship in Ouagadougou, Burkina Faso, on public health strategies to prevent severe malaria in early childhood. In 2009 she obtained her medical degree and started specialization training in psychiatry at the Radboud University Medical Center in Nijmegen. During her training she developed a special interest for geriatric psychiatry. In 2011 she obtained an internal grant from the Radboud University Medical Center to combine her clinical work with a PhD program at the Donders Institute. During her specialization training she was a member of the board of the association of residents at Radboud University Medical Center as well as a student representative within the board of the department for hospital and liaison psychiatry of the Dutch Psychiatric Association. In 2015 she completed a two-month clinical internship in liaison and geriatric psychiatry at the university hospital of Grenoble, France. In the summer of 2017 she obtained a grant together with two colleagues to do stress research at the Lowlands Festival. She finished her specialization training in October 2017. As of January 2018 she will start working as a psychiatrist at the Elisabeth-TweeSteden Ziekenhuis in Tilburg, where she will also continue her research in collaboration with the Radboud University Medical Center and the Donders Institute. Daphne lives with Julien and their three beautiful boys Florent (5), Xavier (3) and Louis (1).

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists.

To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrolment of the best and most motivated students.

The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc. Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy.

For more information on the DGCN as well as past and upcoming defenses please visit:

http://www.ru.nl/donders/graduate-school/phd/.

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